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# THE LIFE OF THE CELL

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# THE LIFE OF THE CELL

*By*

DAVID LANDSBOROUGH THOMSON,

M.A., B.Sc., Ph.D.

LECTURER IN BIOCHEMISTRY, MCGILL UNIVERSITY, MONTREAL

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# PREFACE

It is the aim of this little book to consider in its different aspects the conception of the cell as the unit of life. Its endeavour is to tell the story of the cell in the light of recent advances, which have been many ; and furthermore to show how the new understanding of the cell throws light on each of the biological sub-sciences in turn : histology, biochemistry, biophysics, physiology, embryology, and genetics.

Limitation of space and the desire for the fullest possible integration of the material have frequently compelled the author to treat debatable hypotheses as if they were unchallenged, and to do rival theories the injustice of omitting them altogether ; for these and other faults he must simply make his apology, with the hope that the marshalling of the results of a large body of modern researches on the cell may in some measure justify itself by being useful.

D. L. T.

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# THE LIFE OF THE CELL

## CHAPTER I

### THE VISIBLE STRUCTURE OF THE CELL

*Every animal appears as a sum of living units, each of which bears in itself the complete characteristics of life.*

INTO this short sentence Virchow compressed the truth which was the foundation of the cell-theory of Schleiden and Schwann ninety years ago (1838), and remains the foundation of this little book to-day. All the higher animals and higher plants are built up of a multitude of cells, each of which has so great a measure of independence that the cell may justly be regarded as the microcosmic unit of life. Each individual life begins as a single cell, which divides and subdivides into many daughter-cells, which grow and differentiate and divide until the whole complex structure of the parent is at last reproduced. All the diverse forms of life which we know to-day have evolved step by step,

throughout the tremendous vista of the geological past, from some primitive ancestor akin to the simplest existing animals and plants—so simple and so small that we may regard them as single, wholly independent cells.

We turn naturally to these primitive creatures for an example of the most generalized type of cell, and best perhaps to one of the lowliest of all forms of animal life, the amœba : a tiny speck of living jelly, a hundredth of an inch in length ; constantly changing in shape in the most protean way, with no permanent " front " end, nor any definite " upper " and " lower " surfaces ; moving in a way which deceptively looks simple, by merely flowing in one direction or another ; engulfing particles of food at any point on its surface ; multiplying by dividing into two similar halves. Even the most critical microscopic examination, aided by the best methods of preparation, reveals but little : the living jelly, or protoplasm, is crowded with tiny solid granules and fluid droplets, and contains one most important differentiation, the nucleus, a central, spherical body of the same viscous consistency, in which a more complex internal structure may be deciphered.

In the human body there are examples of cells no less generalized in structure or function than the amœba—the white phagocytes of the blood, which at one time are swept along in the stream,

at another creep out of the blood-vessels altogether, and which surround and if possible digest foreign bodies or invading microbes. The red blood corpuscles, on the other hand, are very highly specialized for their one function of carrying the red colouring-matter hæmoglobin, which combines loosely with oxygen in the lungs and bears it to all parts of the body ; these tiny disc-like cells have lost their nuclei, lost the power of actively moving or changing shape, and indeed lost all but the last vestiges of the signs by which we recognize life. Less markedly altered are the epithelial cells, which lie side by side in a mosaic to form such even surfaces as that of the skin or those lining the alimentary canal and the blood-vessels ; or the gland cells, whose function it is to form and to discharge special substances such as those that make up the digestive juices ; or the cells of the connective tissues. Muscle cells, again, are of two kinds : the one like attenuated spindles, long elastic threads with the nucleus in a slightly expanded section in the middle ; while in the more vigorous *striated* muscle, there are several nuclei, or there has been a fusion of neighbour cells to form compound multinucleate fibres. Nerve cells have typically a central portion of irregular shape, the origin of many short, branching tendrils, and of one main fibre, slender and often very long. By means of these processes the

nervous communications throughout the body are maintained. But what miracles of special-

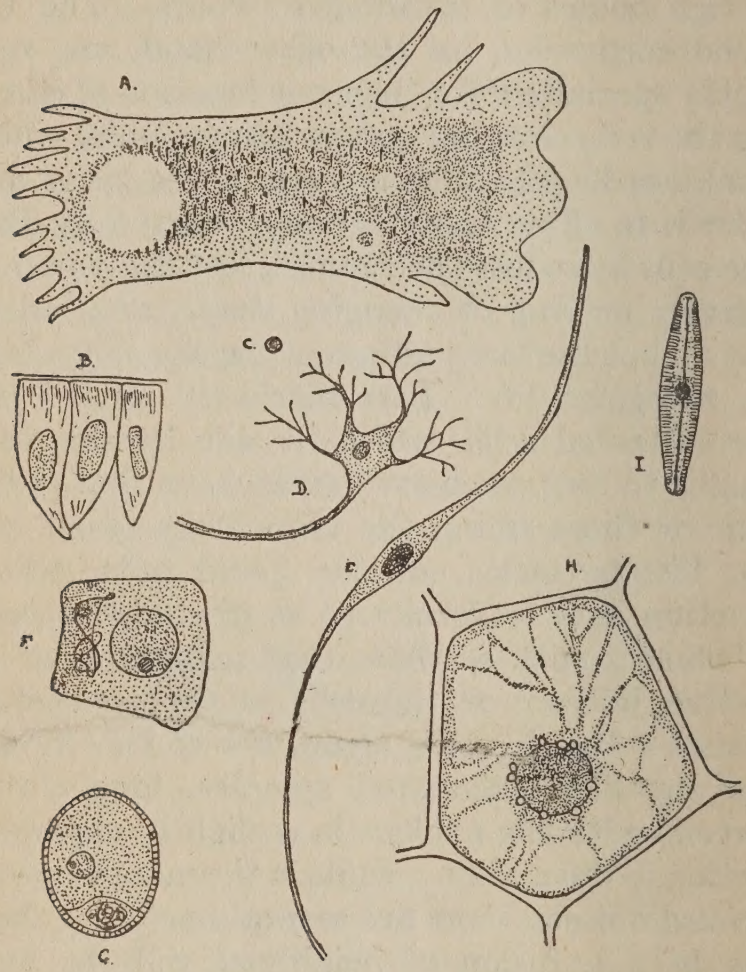



FIG. 1. Cells of different kinds, drawn to one scale (250 times natural size).

A, an Amœba; the cytoplasm is crowded with granules and vacuoles, the nucleus is not shown. B, epithelial cells paving a flat surface, showing irregular nuclei and mitochondria. C, a red blood corpuscle. D, a nerve cell, with branching tendrils and one main fibre or axon. E, a smooth muscle cell. F, a gland cell showing Golgi apparatus, nucleus and nucleolus. G, a grain of pollen, with two nuclei. H, a cell from the surface of a leaf, showing cellulose cell-wall, many vacuoles, and plastids surrounding the nucleus. I, a unicellular plant (diatom) with a flinty shell.

ization are invisibly concealed in the apparently simple structure of the nerve cells that are the seat of memory and of thought, we cannot say ; nor can we tell, except by shadowy clues, *where* in the round, almost homogeneous egg-cell there lies latent the power to reproduce, with exactest fidelity, every character and lineament of the parent.

*The cytoplasm, otherwise homogeneous, typically contains certain definite structures, both living and non-living.*

The *cytoplasm* is the name given to that part of the living protoplasm which lies outside the nucleus. It is bounded externally by an invisible and impalpable film whose existence is deduced from its property of allowing certain substances to pass through, while barring the way to others ; this will be considered in a later chapter. Outside the living matter altogether, there may be some sort of protecting or supporting shell ; most plant cells, for instance, are enclosed in a kind of box of cellulose, and many of the one-celled animals (Protozoa) form temporary or permanent external pellicles as a protection.

Usually the cytoplasm is somewhat heavily charged with inclusions of all sorts ; but where these are absent or have been got rid of, the living matter appears perfectly clear, colourless,

and structureless. Thirty years ago, cytological research was concerned almost solely with cells which had been specially prepared for microscopic examination, by being "fixed" or killed and solidified with powerful chemical reagents, hardened and often distorted by immersion in alcohol, clove oil, paraffin and other media, and stained with artificial dyes. The study of such material is relatively easy and often very fruitful, since minute details can be distinguished, but it may be misleading: for example, fixed protoplasm tends to set in networks to which at one time much importance was attached, but which, it is now generally admitted, correspond to nothing that exists in the *living* cell. In recent times much more care has been taken to check all discoveries made on prepared material by examination of living cells. The study of living cells has been greatly advanced by the elaboration of the methods of "tissue culture," which make it possible to remove a group of cells from a body and keep them alive and under constant microscopic observation, in suitable nutrient media, for weeks and even years; and that not merely in a state of survival, but in perfect health and activity, and exhibiting active movement, growth, and division. Yet another check on methods of microscopic observation is furnished by the introduction of micro-dissection, in which a cell, while remaining all the time under

the microscope and watched by the experimenter, can be probed and manipulated with extremely fine glass needles whose movements are controlled by delicate screws. Bearing in mind the results obtained by these different methods, modern workers are now fairly well agreed that living matter is a slightly viscid fluid, varying indeed in the degree of its viscosity from cell to cell, or from one time to another in the same cell, and entirely without structure except such as depends on the presence of the various inclusions.

Of these, we may consider first the recognizable reserve stores, which are plainly not to be regarded as forming part of the truly *living* matter. Such, for instance, are the starch grains to be seen in many plant cells, and the corresponding grains of glycogen in a few animal cells. Many animal cells also contain droplets of fat or oil, and egg-cells in particular are often heavily charged with yolk. The chemical significance of these substances will be made plain in the two succeeding chapters. Gland cells, whose function it is to elaborate and secrete special substances, are often crowded with granules to an extent partly depending on their degree of activity ; so that overworking of the gland causes a disappearance of the granules, which may be regarded as raw materials or unfinished products. In addition to these in-

clusions which are either somewhat solid or consist of a liquid immiscible with water, there may also be large and small droplets of aqueous solutions ; and these, it is reasonable to suppose, are bounded by protoplasmic films similar to that which forms the external boundary of the cell (since otherwise the water would certainly diffuse into the protoplasm, just as a cell would probably melt away in water if it were not for the external membrane) ; such droplets are called " vacuoles." In the amœba, for instance, vacuoles form round particles of food as soon as they are ingested ; and other large vacuoles may be seen to shrink and swell in rhythmical pulsation. In the Protozoa, indeed, vacuoles may be so numerous as to give to the whole of the cytoplasm a foam-like appearance ; and in the higher plants, likewise, the protoplasm may form but a tenuous framework within the box-like cellulose wall, the rest of the space being taken up by large vacuoles.

Passing on now to consider those inclusions which, while in one way or another distinguishable from the typical clear cytoplasm, may yet be regarded as being composed of *living* matter, we may take first the " plastids," which are especially characteristic of plant cells ; in their simplest form these are small, colourless, often rounded bodies, which may be regarded as the seat of definite chemical reactions within the

cell: thus a plastid may be the focus around which a starch-grain is built. In other cases the plastid becomes charged with some pigment or other, and notably by chlorophyll, the green colouring-matter of plants. These chloroplasts are undoubtedly the locus of the reactions by which the green plants utilize the energy of the sunlight to build up complex chemical substances, as will be seen later. There is now considerable evidence that plastids grow in size at the expense of the cytoplasm and multiply by division; it is not unlikely that all plastids arise—however they originated in the first place—by the division of previously existing and similar bodies.

Similar considerations apply to cytoplasmic inclusions of another type, the "mitochondria" (also called chondriosomes, and by many other names, unfortunately!), which also grow and multiply, and probably never arise *de novo*. These structures, very widely distributed in cells of all kinds, are rather small granules or delicate threads, constantly in motion and constantly changing in shape from the granular to the filamentous. They may be made plainly visible in living cells by treatment with dilute solutions of a special stain (Janus green B), which has a peculiarly selective action on them, but, as in the special methods employed for demonstrating them in fixed and stained prepar-

ations (the ordinary methods often dissolve away the mitochondria altogether) a possibly distorted and certainly unduly solid appearance is given to these delicate structures, there is no evidence that they are less fluid than the surrounding cytoplasm. Of the function of the mitochondria there is at once too much and too little to say : there is hardly a chemical reaction known to take place within living cells with which the mitochondria have not been correlated by one investigator or another. It is by no means impossible, however, that the mitochondria (and more particularly their surfaces, as we shall see later) are regions of peculiarly intense chemical activity, even if their own substance does not enter into the reactions.

Even less satisfactory is our knowledge of another formed component of the cytoplasm, usually called the " Golgi apparatus." This, in suitably prepared material (it is, unfortunately, almost impossible to see in living cells), appears as a rather dense network, about the size of the nucleus of the cell, and resembling a partly unravelled knot in a piece of string. In some of the lower animals and in the Protozoa, it appears to be represented by scattered granules ; it is rather doubtful if it occurs in plants at all. The best thing that can be said for the Golgi apparatus is that in certain types of cell it occupies a definite position with great constancy :

thus in gland cells it lies between the nucleus and the surface through which the cell must (from anatomical considerations) be presumed to pour out its products ; there is, indeed, a fair amount of evidence that the Golgi apparatus is concerned with the discharging of substances, whether useful compounds or waste products, from the cell. Chemically, the Golgi apparatus appears to be somewhat similar in composition to the mitochondria, and physically little less (perhaps considerably *more*) fluid than the surrounding cytoplasm. The evidence that the Golgi network in each cell arises only by the division of the network in the parent cell is not as clear as could be wished.

Finally, we may briefly mention the " central body," which is to be found in the cells of most animals and the lower plants. This typically consists of a minute granule, the centriole, which may be surrounded by a definite sphere of protoplasm, the centrosome. The central apparatus undoubtedly plays an important part in the division of cells, and it may be discussed more advantageously in that connection.

*The most constant and most important structural feature of the cell is the nucleus.*

The nucleus is usually a body of rounded shape, completely surrounded by the cytoplasm, from which it is separated by a thin proto-

plasmic film. In the living cell, except when division is in progress, the interior of the nucleus appears to be quite fluid and structureless apart from the presence of one or more denser, spherical "nucleoli," which are probably aggregates of stored material or waste products. In fixed cells, on the other hand, it regularly displays a net-like structure, produced by the coagulation of the fluid living matter. The protoplasm of the nucleus is peculiarly sensitive, and can be caused to coagulate in this way by slight chemical or mechanical injury.

Although the appearance of the nucleus in fixed and stained preparations is doubtless unnatural, it is nevertheless instructive, and deserves some attention. There is usually distinguishable a fine network, on the strands of which are scattered dense granules which are very deeply coloured by certain stains and are supposed to consist of a substance called "chromatin" from this property. Chromatin is deeply stained by the natural dyes carmine and hæmatoxylin, and by the basic coal-tar dyes such as safranin, toluidine blue, and gentian violet. A "basic" dye is one which is a combination of a complex organic base with a simple inorganic acid, whereas in an "acid" dye the base is inorganic and the acid complex and organic. The acid dyes, such as eosin, have little affinity for chromatin but more for the

other constituents of the nucleus and for the cytoplasm; other acid dyes—fuchsin, methyl blue—have a special tendency to stain the strong, elastic protoplasm of muscle and connective-tissue cells. It is very doubtful whether the staining reactions of any part of the cell can tell us much of its chemistry; recent work suggests that physical factors, such as density and electric charge, are of greater importance in staining than chemical composition: acid dyes are said to soak into the substance of the less dense portions of the cell, basic dyes to precipitate upon the surface of the more compact elements (it will be borne in mind that these remarks apply to staining *subsequent* to fixation). It is, however, likely enough that chromatin consists in part at least of substances of a well-defined type. There may often be found in the cytoplasm granules or rods which show much the same staining reactions as chromatin, and hence called " chromidia " and said to be derived from the nucleus. This view will be received with least doubt in the case of certain one-celled animals and plants (bacteria, for instance), which apparently have no definite nucleus but an abundance of chromidia whose behaviour certainly suggests that they are representatives of the missing organ.

These cases apart, the wide distribution and functional importance of the nucleus in living

cells can scarcely be over-emphasized. If the nuclear membrane be gently torn, so that the contents escape into the cytoplasm, they produce therein an immediate disturbance and liquefaction ; Chambers has shown that if this is done to one half of an amœba which has almost completed the process of division, so that the two daughter-cells are connected only by fine strands of protoplasm, none the less the disturbance spreads across into the uninjured half and causes the coagulation and death of the nucleus there. A fragment of cytoplasm cut away from the cell, and containing no nuclear material, may indeed survive for a time, but it has lost many of the properties of the complete cell. For instance, if the single-celled animal *Stentor* be cut into several fragments, those pieces which contain a part of the nucleus will regenerate and form complete new individuals, while those without nuclear material cannot regenerate and eventually perish. Most significant of all is the fact that a cell without a healthy nucleus cannot carry out the complex process of cell-division under any circumstances whatever.

*Cells are formed only by the division of pre-existing cells, typically in a complex, equal, and orderly manner.*

If the first great premise of the cell-theory was, in Schwann's words, that " all organized

bodies are composed of essentially similar parts, namely, of cells," then the second was undoubtedly that stated at the head of this section, or, in Virchow's phrase, *omnis cellula e cellula.* Not till about 1880 was the corollary added: nuclei arise only from pre-existing nuclei; but to-day, as we have seen, similar statements are freely made of the origin of the mitochondria, the Golgi apparatus, the centrioles, and even the plastids.

The first stage in typical cell-division is the division of the centriole into two parts, which slowly move apart until they are lying at opposite ends of the cell with the nucleus between them. As they move, they begin to show a peculiar effect on the cytoplasm which becomes more marked when the final positions are attained: around each centriole the living matter, as is shown by the minute granules and vacuoles therein, arranges itself radially, to present the appearance of a star or "aster." Ultimately almost the whole cytoplasm is filled by the rays of these two asters, which may indeed interpenetrate; and at the same time the protoplasm becomes abnormally dense, except in the centre of each aster and at the extreme periphery, where it is more fluid than ever.

Meanwhile changes go on within the nucleus—the "prophase" of the division of the nucleus,

which, when it is of this usual type, is a process called "mitosis" or "karyokinesis." There appears within the nucleus a winding thread, at first perhaps continuous but soon broken into segments, on which the chromatin granules are accumulated: it is called the spireme. From a very early stage the thread is double throughout all its length—a hint of the equal division to come. It is true that it may be impossible to distinguish this structure without fixing, or at least injuring, the nucleus: but in the later stages of mitosis the appearance of fixed specimens so exactly corresponds with the living reality that one is less than usually suspicious of the preparations in this instance.

The separate sections of the double spireme thread now become progressively shorter and thicker, till they resemble more or less stumpy rods or even ovals, and the double structure may be temporarily obscured. The bodies thus formed are largely composed of deeply-stained chromatin, and are called "chromosomes"; we shall see in a later chapter with what an important rôle modern cytology invests them.

In the "metaphase" the chromosomes, previously lying scattered at random in the nucleus, begin to arrange themselves in a definite way. At the same time the nucleus becomes more oval, and the membrane disappears, so that some of the nuclear material probably escapes into the

cytoplasm. A great part of the nuclear material, however, goes to form a structure which from its shape and its resistance to staining processes is called the "achromatic spindle." The ends of the spindle are close to the centres of the asters, and at the widest part, the equator, the chromosomes arrange themselves in a plane; their duplex structure becomes more manifest, and finally each splits into two separate and similar bodies.

The cell as a whole becomes longer and narrower, and the asters move somewhat apart; and of each double chromosome half moves with one aster and half with the other. Then, as the "anaphase" sets in, the separated chromosomes begin to draw nearer to the centres of the asters, that is to the apices of the achromatic spindle. In doing this, they become somewhat deformed, into U-shapes and J-shapes: exactly as soft, inelastic bodies would be deformed if pulled through a resistant medium by a fibre attached to the centre (U-shape) or near to the end (J-shape) of their lengths. Micro-dissection tells us that the spindle is fairly dense in consistency, during the metaphase, but that the space left between the two sets of chromosomes as they separate is filled with fluid protoplasm; it tells us also that the chromosomes are relatively solid bodies; and it is with something of a pang that we confess that it gives no hint of

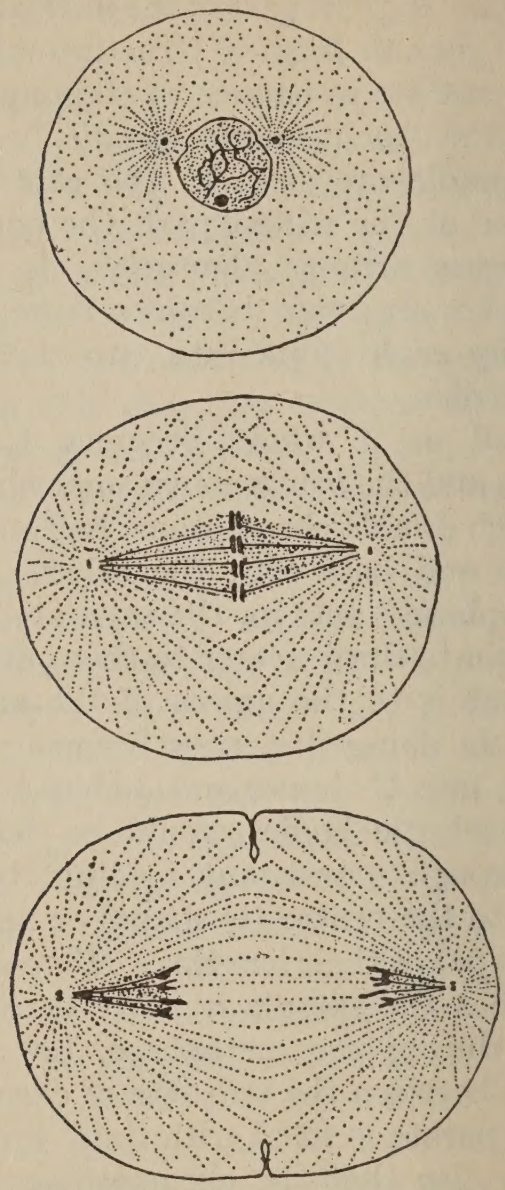


FIG. 2. Cell division : prophase, metaphase, and anaphase.

the presence of fibres drawing the chromosomes towards the ends of the spindle. It remains to add that in spite of the enormous amount of study, thought, and imagination that has been devoted to the problem, we have absolutely no knowledge of the forces that bring about the formation of the asters, and control the movements of the chromosomes.

Finally, in the "telophase," the chromosomes of each of the two groups lose their regular outlines, branch, and join to form a network whose structure becomes ever less definite: and round each group a new nuclear membrane forms. We have now two daughter-nuclei, one at each end of the cell, with the chromatin of the parent nucleus exactly divided between them. At each end of the cell there is also a centriole, or rather two, for the centriole often splits in preparation for the next cell-division before the previous one is fully accomplished. The last stage is the division of the cytoplasm: the cell is by now decidedly elongated in the direction of the axis of the spindle, the line joining the two division-centres. In the plane at right angles to this there appears on the surface of the cell a girdling furrow, which sinks more and more deeply in until the two halves are connected only by a thin waist; this in turn disappears, and the two daughter-cells are now completely and irrevocably separated. It is

hard to see with how much exactness the cytoplasm is divided between the two halves, hard even to follow the partition of the mitochondria, except in a few exceptional cells where the mitochondrial material is concentrated into a few dense masses ; in these cases the masses can be seen to gather at the " equator " of the dividing cell, and to be evenly distributed to either side. The Golgi network is said to split up into separate fragments, which are divided between the daughter-cells in a similar manner.

In the higher plants generally, although the formation of the spindle and the division of the nucleus goes on in exactly the same way, the division of the cytoplasm takes place rather differently. In the first place there are no centrioles, and consequently no asters. In the second place, the two cells are not constricted off from each other by an encircling furrow, but by the building of a wall between them, commencing with a deposition of material to form a plate at the equator of the spindle ; this plate ultimately grows right across the cell. It would seem that the partition thus formed between the daughter-cells is not perfectly continuous, for connecting " bridges " of protoplasm from cell to cell are frequently observed in plant tissues.

A slightly different (" heterotypic ") form of nuclear division, of the same complex type,

occurs in certain well-defined cases and will be considered in connection with the life-history of the germ cells. Nuclear division without the elaborately organized distribution of components is also known to occur ; it is called " amitosis," and is probably always a sign of degeneracy or unhealthy conditions ; it is not necessarily followed by a division of the cytoplasm. This concludes the survey of the visible structure of the cell.

## CHAPTER II

### THE CHEMICAL STRUCTURE OF THE CELL

*Many chemical elements are represented in the complex mixture of substances which constitutes living protoplasm.*

Of the known chemical elements, at least a quarter, that is to say over twenty, have been reported, at one time or another, to be present in living tissues. But a number of these can be dismissed immediately, for they are probably present by accident only, and have nothing to do with the chemistry of life. Several others, too, cannot be dealt with in these pages: for even if they do play an important part in the life of the cell, that part is still too obscure to be discussed here. There remains the following short, irreducible list: *carbon*, *hydrogen*, the two essential components of the so-called "organic" substances, from whose combustion the energy of living matter is derived; *oxygen* and *nitrogen*, the two gases of which the air we breathe is a mixture; *sulphur*, *phosphorus*, *chlorine*, and the metals *sodium*, *potassium*, *cal-*

*cium, magnesium, iron.* Now since about 80 per cent. by weight of living matter is *water*, which everybody knows to be a compound of hydrogen and oxygen ($H_2O$), and as the gases of the atmosphere are capable of dissolving in water to some little extent, we find, as we should expect, that living matter contains dissolved the element oxygen and the element nitrogen. But, with these exceptions, the elements mentioned above are not present in the free state, but occur as chemical compounds of one element with another: two atoms of the gas hydrogen uniting with one atom of the gas oxygen to form one molecule of water being perhaps the simplest case, and one which illustrates as well as any other how little the properties of elements may be reflected in the properties of their compounds.

Since water itself constitutes so large a fraction of living matter, and since the other substances present are either uniformly dissolved in the water, or, if they do not dissolve, so intimately mixed with the water that the consistency of the whole is that of a thin, fine-grained paste, it is clearly desirable to devote some study in the first place to the properties of water itself; secondly to the properties of *solutions* of substances in water; and thirdly to the properties of intimate mixtures of insoluble substances with water To these physical problems, as

well as to the purely chemical problems of th structure and properties of the compounds which living matter is composed, this chapt will be devoted. For the living cell is so com plex an organization, physically and chemicall that its peculiar powers and properties cannc be understood without a grasp of many impo tant chemical and physical principles.

Let us begin with the simplest possible cas the consideration of the state of matter in a ga such as oxygen. The gas consists of tin independent "molecules," all alike; each is i rapid motion, but cannot proceed far withou colliding with another; the two molecules re bound elastically, with no loss of energy, an dash off at an angle to further and furthe collisions; the constant succession of such tin impacts on the walls of a containing vesse produces the "pressure" of the gas. If th gas is compressed, as in a bicycle tyre, th molecules are brought nearer, the impacts ar more frequent, the pressure is higher. In liquid, the condition is similar, except that th molecules are more closely packed, and cannc well be compressed; the movements, too, ar less active. But it is quite easy to demonstrat that this ceaseless agitation does go on, fo although the molecules themselves are far toc small to be made visible, nevertheless particles suspended in the liquid, and large enough to be

een under the microscope, share to some extent
n this agitation, and can be seen to dance
estlessly hither and thither. This is called the
'Brownian movement," after its discoverer,
nd can be observed in living matter—a further
vidence of the fluid nature of most protoplasm.
Even in solids, there is a ceaseless oscillation
o and fro of the molecules, bound in definite
laces though they are. All these movements
re influenced by temperature—the higher the
emperature, the more violent the movement:
r perhaps it would be better to turn this sen-
ence around, and define a high temperature as
he condition in which molecules are unusually
ctive.

From this consideration of the "kinetic
heory" of a pure gas, a pure liquid, and a pure
olid—whether chemical elements or chemical
ompounds, in which latter case the molecule
ontains dissimilar atoms—let us turn to solu-
ions and mixtures. All gases mix readily: the
nolecules of one wander freely among the mole-
ules of the others. Similarly, two liquids may
nix, the one dissolving in the other; but in
ther cases the molecules of one may be unable
o penetrate among the molecules of the other—
ve cannot say *why*—and the liquids, like oil and
vater, do not mix. In the same way some solids
re unable to send their molecules out wandering
mong the molecules of water, and, like sand,

they do not dissolve ; but others, such as common salt (sodium chloride), dissolve readily, and the molecules of salt travel to all parts of the solution. The properties of such a solution are of importance in these studies, and merit some attention.

Consider, as a concrete case, a dish containing water, covered by a large bell-jar. If the air in the bell-jar is dry, a certain amount of the water in the dish will evaporate, however low the temperature, until the air in contact with it is saturated with moisture. When this state of equilibrium is reached, no further change will be detectable while the conditions are kept constant ; but for all that, a ceaseless circulating activity will be maintained. At every instant molecules of water vapour will be condensing on the surface of the liquid ; but this is exactly counterbalanced by the ceaseless escape into the air of water molecules from every point on the exposed surface. The degree of vigour with which water molecules escape from the surface of the liquid is termed the " vapour pressure," and this will plainly be a measure of the degree of saturation with moisture of the surrounding air, when equilibrium has once been established and loss exactly balances gain. The vapour pressure increases with the temperature, so that as the liquid becomes hotter more and more molecules will escape or evaporate,

until finally the vapour pressure becomes equal to the barometric pressure of the air, and the water *boils*. Pure alcohol has a higher vapour pressure than water, and in consequence evaporates more quickly and boils at a lower temperature.

Let us now suppose the water to be replaced by a solution of salt in water. It is plain that a certain percentage of the surface area of the liquid will be occupied by molecules, not of water, but of salt, which cannot escape in the form of vapour. Consequently water molecules will only be able to evaporate from the remaining fraction of the surface, instead of from the whole area: at a given temperature the vapour pressure of the solution will therefore be less than that of pure water, and it will require a higher temperature to bring the solution to the boiling-point. The decrease in the vapour pressure is plainly proportional to the relative number of non-volatile dissolved molecules in the solution—the molecular concentration.

Suppose now that we line a porous pot with a continuous membrane of copper ferrocyanide, for example, which is semi-permeable: that is, permits water molecules to pass through, but prevents the passage of the molecules of salt and many other dissolved substances. Then let the pot be filled with salt solution, and immersed in pure water; the molecules of water,

being in ceaseless movement, will travel to and fro through the membrane. But whereas outside the pot water molecules are striving to pass the membrane at all points on the surface, inside the pot a part of the surface is blocked by molecules of salt which are unable to pass through ; the stream of water into the pot will accordingly be greater than the corresponding stream out of the pot, and the volume of solution within the pot will increase. This will continue, even if it entails a steady piling-up of pressure within the pot, until a point is reached when the driving-power of the internal pressure is sufficient to compensate for the smaller area available for water molecules leaving the pot, when equilibrium is established, and no further change takes place. The pressure within the pot is then spoken of as the *osmotic* pressure of the solution, and is clearly proportional to the molecular concentration. It is plain that a close connection between vapour pressure and osmotic pressure exists, since it is reasonable to consider the surface of a solution in contact with the air as a membrane which is permeable to water molecules but not to the molecules of dissolved salt.

Such considerations lead us to the important conception of the analogy between a dissolved substance and a gas. If a small volume of a gas is set free in a large space, then, since

the gas molecules are in constant and violent motion, diffusion will take place until the gas is equally distributed throughout the whole space ; it matters nothing if the space is already occupied by other gases, diffusion will take place, though more slowly, until equal distribution is obtained. In the same way the molecules of a dissolved substance, being similarly in incessant motion, will diffuse throughout the whole available volume of solvent, as quickly as the greater resistance of a liquid permits, until equal concentration in all parts of the solution is attained. The osmotic pressure of a solution upon a semi-permeable membrane can therefore be compared to the pressure of the internal gas upon the walls of an impermeable balloon. It may be added that the analogy is quantitatively as well as qualitatively accurate.

*Certain molecules dissociate when dissolved in water.*

The theory outlined above demands that solutions of equal molecular concentration should have the same osmotic pressure and vapour pressure. For example, the molecular weight of cane-sugar being 342, and that of urea 60, a solution of 342 grams (one "gram-molecule") of cane-sugar in a litre of water should have the same osmotic pressure as a "molecular" solution of urea, i.e. 60 grams

in the same volume : this is, indeed, found to be the case. But when with these solutions is compared a molecular solution of sodium chloride (59 grams per litre), it is found that the salt solution has an abnormally high osmotic pressure and correspondingly low vapour pressure. The three solutions also differ in another important point, that the salt solution will conduct an electric current, while solutions of cane-sugar and urea will not.

These facts are explained by the theory, nowadays elevated to the status of a general law, of *electrolytic dissociation*. When sodium chloride is dissolved in water, a very large number of the molecules break up into two parts, called *ions*, of sodium and chlorine respectively. An ion may be defined in this case as an atom carrying an electric charge—a positive charge on the sodium ion, and an equal negative charge on the chlorine ion ; but, as we shall shortly observe, an ion may be a group of several atoms. Electrical charges of the same sign repel one another, so that the sodium ions do not group together to form recognizable masses of sodium ; and electric charges of different sign attract one another, so that it is impossible to separate the sodium ions from the chlorine ions (without adding other substances or removing the electric charges), or to have a solution with more positive than negative charges in it, and so on. All ions

carry either the same unit charge of electricity, positive or negative, or some simple multiple of this unit.

The bearing of this theory on the difficulties suggested in the paragraph before can readily be followed. Clearly the electric current is transported through the liquid by these electrically-charged ions, the sodium ions carrying the positive current and the chlorine ions the negative. Secondly, since the copper ferrocyanide membrane is no more permeable to the ions than it is to the undissociated molecules, it is obvious how the abnormally high osmotic pressure arises. If, instead of a given number of dissolved molecules striking against the membrane in their endeavours to get out into a greater space (for this, as we have seen, is a justifiable way of regarding osmotic pressure), we have almost twice that number of ions, we shall clearly have a great increase, almost a doubling, of the osmotic pressure—since the size of the dissolved particles is of no account in comparison with their number.

Many familiar reactions take place between ions, and depend on the fact that a ceaseless interchange between the ionic and the molecular states goes on : there is a constant dissociation of the intact molecules which still remain in the solution, balanced by a constant fortuitous union of ions to form undissociated molecules.

If, for instance, a solution of silver nitrate is prepared, the ions formed are silver (positive) and nitrate (negative—an ion consisting of four atoms, but bearing one unit electric charge). Let this solution be added to the sodium chloride solution ; then, as the ions of opposite sign come together fortuitously, molecules of silver chloride will be formed ; silver chloride being insoluble in water, these molecules will aggregate to form a solid sediment, and, since they are not dissolved, will no longer form ions. Sodium nitrate molecules will also be formed, but remain dissolved, and soon split up again into ions, whereas the formation of solid silver chloride, being irreversible, will go on until all the silver or all the chloride is used up—both, if they are in equal atomic proportions, at the same moment, so that only sodium nitrate, largely dissociated, is left in the solution. Reactions between ions are characterized by their great rapidity.

Compounds which in solution form ions are termed electrolytes ; they are divided into three classes—acids, bases, and salts. An acid is a compound dissociating to yield positive ions of *hydrogen* : the concentration of hydrogen ions in a fluid is the measure of its acidity. A base tends to fix hydrogen ions, to remove them from the solution ; it may be, for instance, a compound yielding negatively charged *hydroxyl* (oxygen plus hydrogen) ions, and for the moment

we may regard the concentration of hydroxyl ions in a fluid as the measure of its alkalinity. Acids combine with bases in the following way: the hydrogen ions are fixed by combining with the hydroxyl ions to form molecules of water ($H_2O$), and the negatively charged ion of the acid combines with the positively charged ion of the base to form a molecule of a typically neutral *salt*; but salts, if soluble, are generally much dissociated in solution. For example, hydrochloric acid and sodium hydroxide react to form water and sodium chloride:

$$HCl + NaOH = H_2O + NaCl.$$

Electrolytes vary greatly in the degree to which they dissociate into ions. Thus in dilute solutions sodium chloride, hydrochloric acid and sodium hydroxide are almost wholly ionized, while only a small proportion of the molecules of acetic acid or the base ammonia dissociate: the degree of dissociation is a measure of the strength of an acid or a base.

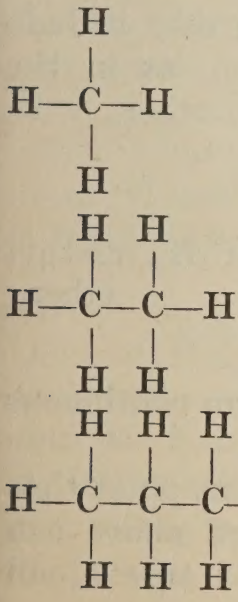
The purest obtainable water dissociates to a very slight extent into hydrogen and hydroxyl ions; as the number of each kind is inevitably equal, the liquid is neither acid nor alkaline but exactly neutral; at ordinary temperatures it contains one gram weight of hydrogen ions in ten million ($10^7$) litres volume. Any solution containing a greater concentration of hydrogen

ions than this is acid; any solution containing a less concentration is alkaline, for it must have a greater concentration of hydroxyl ions than neutral water if the hydrogen ions are so few; as the one increases the other decreases, so that the product of the two is constant. For biological purposes acidity and alkalinity are habitually measured in terms of hydrogen ion concentration, and usually expressed as *pH*; thus neutrality is expressed as *pH* 7 (approximately). An alkaline solution containing only one gram-ion of hydrogen per thousand million ($10^9$) litres volume is of *pH* 9, and an acid solution containing a gram-ion of hydrogen per hundred ($10^2$) litres has a *pH* of 2.

*The compounds of carbon, "organic" compounds, are more numerous, and very much more important in living matter, than compounds of all the other, "inorganic," elements put together.*

Carbon differs from all other elements in the number and variety of the compounds which it forms; so much so, that the study of these compounds is considered a distinct branch of chemistry, called "organic" to mark its close connection with the composition of living organisms. There are two reasons for this property of the carbon atom, and one is that it is an atom of high "valency," having the power

to unite with four atoms of hydrogen (or to replace one or more of these with various other univalent atoms, such as chlorine, or univalent groups such as hydroxyl), or to combine with two atoms of a bivalent element such as oxygen. There are, however, not a few atoms whose valency is equal to or greater than that of carbon, and we accordingly place greater significance in the second and more unique property, the power of carbon atoms to join together to form chains. In the middle of such a chain each carbon atom has two of its four valencies occupied, one connecting it to each neighbour, while the other two unite it to atoms of hydrogen or other suitable groups. In this way we get compounds of the following type :

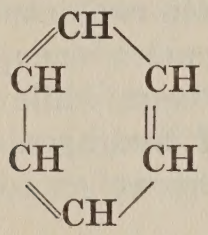


$CH_4$, methane or marsh-gas.

$C_2H_6$, ethane, also written $CH_3.CH_3$.

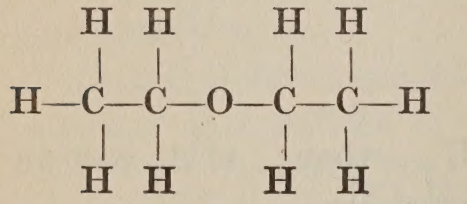
$C_3H_8$, propane, also written $CH_3.CH_2.CH_3$.

In this way enormously long chains of carbon atoms with their attached groups may be formed. Moreover, the chains need not necessarily be straight, but may be branched, one carbon atom at the "junction" being linked to three or even four neighbouring carbon atoms. Often, too, the chains may curve "round" (as far as these formulæ are any adequate representation of reality) and make a closed chain or ring, such as the well-known benzene ring:



$C_6H_6$, benzene.

In other cases the chain or ring may include links of other atoms than carbon, as in the formula of the familar anæsthetic, ether:

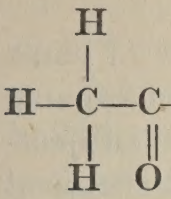


$C_2H_5.O.C_2H_5$, diethyl ether.

but only the carbon atoms can form continuous chains.

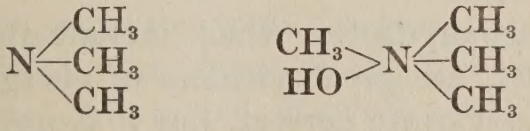
It may be as well to remark at this point that none of the compounds formulated above can form ions, but this does not hold true of all

organic compounds. There are organic acids, such as acetic acid



$CH_3.COOH$, acetic acid.

which dissociates, setting free as an ion the hydrogen atom of the —COOH group (carboxyl group, typical of organic acids), and which forms well-ionized salts with inorganic bases. There are also organic bases which form salts with inorganic acids; but we seldom find salts of which both ions are organic. It should also be noticed that in organic compounds a hydroxyl group directly attached to a carbon atom, as in $CH_3.CH_2OH$ (ethyl alcohol) is never split off as a negative ion. Typical organic bases derive their properties from the power of the nitrogen atom to have either three or five valencies: thus from tri-methylamine is derived the base tetra-methyl-ammonium-hydroxide:



and all $NH_2$ or *amino* groups are similarly to be regarded as basic. The extra valencies of the nitrogen atom allows the compound to fix hydrogen ions.

*The principal organic compounds present in living cells are conveniently classified as carbohydrates, fats, and proteins.*

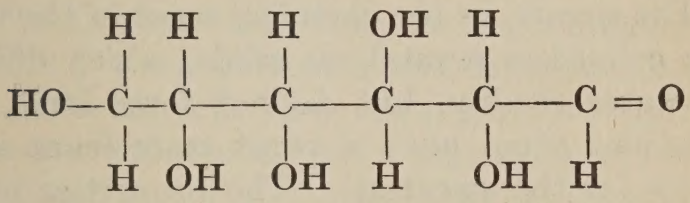
From the foregoing brief discussion of some elementary principles of inorganic and organic chemistry we may turn to the true subject-matter of this chapter, the nature of the chemical compounds present in living cells. Sodium, potassium, and calcium may be briefly dismissed, since they occur mainly as inorganic salts dissolved in the water which, after all, constitutes something like four-fifths of the weight of all living cells ; the chlorine present nearly all goes to form the negative ions of these salts. The iron, on the other hand, is more probably bound into the structure of complex organic compounds : hæmoglobin, for instance, the red pigment of the blood, contains iron.

The more elaborate organic compounds present in living cells are best divided into three groups, namely : carbohydrates, fats, and proteins : but this classification is by no means exhaustive, as we shall see.

The carbohydrates, which include starch and the sugars, are great yielders of energy. It is perhaps not quite correct, but it is nevertheless tempting to regard them as fuel for the machinery of the living cell rather than as part of the machinery itself ; and this view is on the whole confirmed by the fact that in the cell they are

often to be found in solid granules, manifestly stores of chemical energy waiting to be used up; nor does physiology fail to support this position, as will be seen. Chemically, the carbohydrates are fairly well defined: they contain carbon, hydrogen, and oxygen only, and the hydrogen and oxygen are invariably present in the proportions of water ($H_2O$); that is, there are two atoms of hydrogen for each one of oxygen. The number of carbon atoms is typically six, or else twelve, or else some higher multiple of six: though sugars of five carbon atoms are well distributed in the plant kingdom.

Glucose, or grape-sugar, is the most important of the many six-carbon-atom or "hexose" sugars. It has the formula $C_6H_{12}O_6$, or



and consists therefore of a straight chain of six carbon atoms united to hydrogen atoms, hydroxyl groups, and a single oxygen atom. When it is realized that if one of the "H—C—OH" groups in the above formula were written "HO—C—H" instead, an entirely different sugar would be represented, it will be appreciated that there are, in truth, a large number of hexose sugars, similar but not identical.

The most important twelve-carbon-atom sugars are ordinary cane-sugar, milk sugar, and malt sugar. They all have the (abbreviated !) formula $C_{12}H_{22}O_{11}$, and may be regarded as two hexose molecules joined together *minus* a molecule of water. In fact, malt sugar, for instance, can be split up into two molecules of glucose, if water is present. In the same way the complex carbohydrates, such as starch, may be regarded as made up of hexose units, from each of which a molecule of water is subtracted, and have the general formula $(C_6H_{10}O_5)_n$. As it is not known what the size of these complex molecules actually is, no definite value can be written for $n$; it is certain only that many hexose units go to make up a molecule of starch.

The sugars, in the chemical sense of the term, are colourless crystalline solids, which dissolve in water readily, but do not form ions; the solutions often have a sweet taste (cane sugar is by far the sweetest). The properties of the complex carbohydrates are rather different, and the three important examples may be discussed separately. Starch, which occurs so widely in plants as a reserve food-store—in potatoes, for instance—is probably not a single chemical compound; it does not truly dissolve in water, but forms "colloidal" solutions—that is to say, "solutions" in which the individual particles are of more than ordinary molecular size, as we

shall presently see ; the same is true of the other complex carbohydrates. Of these it will be enough to mention glycogen, sometimes called animal starch, since it is a very similar substance, and plays in some animal cells very much the same part as starch plays in plant cells ; for instance, it is stored up in the liver and in the muscles. Rather different and more insoluble is cellulose, which we have already mentioned as a chief constituent of the rigid, non-living walls which enclose most plant cells ; it is not quite unknown in the animal kingdom.

Turning now from the carbohydrates to the fats, we are tempted to class these again as "fuel." Every one is familiar with the general appearance of fats, and with some of their properties : they are soft and not usually crystalline ; they melt at a low temperature—in fact in living cells they are generally liquid ; they do not dissolve in water, and when melted they will not mix with it ; and they do dissolve, somewhat readily, in the so-called "organic solvents," liquids like ether, benzene, and the paraffins (we have all used such liquids to get rid of grease spots).

A little preliminary explanation is required before the chemical structure of the fats can be made plain. It has been pointed out that although alcohol ($CH_3.CH_2.OH$) contains an hydroxyl group, it does not form ions and is not

a base ; and consequently the compound of an alcohol with an organic acid is not a salt, but is called an *ester*. Ethyl alcohol combines with acetic acid to form an ester called ethyl acetate :

$$CH_3.CH_2.O—C.CH_3$$
$$\quad\quad\quad

sodium and potassium, and the salts are sufficiently familiar to us as *soaps*. A molecule of fat, then, consists of one molecule of glycerine coupled with three molecules of fatty acid, not necessarily all alike.

There are a number of classes of compounds which are often considered along with the fats for convenience, largely because they have the same insolubility in water and solubility in organic solvents. The " fatty oils," like linseed oil and castor oil, are really fats but happen to be liquid at ordinary temperatures. The " waxes," such as beeswax, lanolin, and spermaceti, are less closely related, and glycerine has no part in them ; the sweet-scented " essential oils " or " terpenes " are not related to the fats at all, except by solubility ; they include clove oil, lemon oil, camphor, and india-rubber. But all these compounds are found only very sporadically in animal or plant tissues.

The term " lipoid " or fat-like substance is usually, however, restricted to a few rather enigmatic substances of much wider distribution and probably deeper significance in living cells than any of the above-mentioned, somewhat specialized substances. The first example is *lecithin*, which resembles the fats in being an ester of glycerol ; but in this case, while two of the hydroxyl groups are combined as before with fatty acids, the third is combined with a

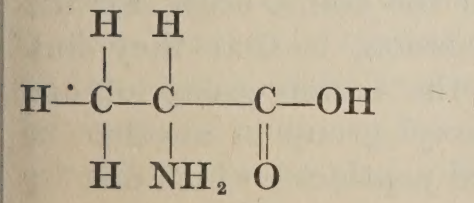
molecule of phosphoric acid, and this in turn links on to a complex, nitrogen-containing base, choline ; it is a white, rather waxy substance, soluble in ether, and forming colloidal solutions in water ; several closely allied substances are known, some of which contain a molecule of sugar. Another example of a lipoid is *cholesterol*, which is an alcohol of complex structure, solid, and similar to lecithin in its solubilities. Important physiological rôles have been ascribed to both these substances, which will require further discussion.

*The proteins are regarded as the most characteristic and essential constituents of living matter.*

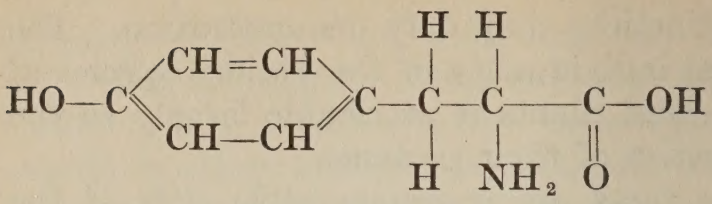
The proteins are sharply distinguished from the carbohydrates and the fats in their elementary composition, since they contain, as well as carbon, hydrogen, and oxygen, invariably nitrogen and usually sulphur. These elements are present in rather constant proportion, so that it was at one time thought that protein was a single substance, though so widely distributed in nature. On the contrary, it is now recognized that the number of different proteins is practically infinite. For example, the three chief proteins of human blood are undoubtedly distinct from the corresponding proteins of the gorilla, and these in turn are different from their analogues in the chimpanzee—though *chemically*

the distinctions may defy demonstration. The chemical individuality of the various species of animals and plants is ascribable largely to the peculiarities of their proteins.

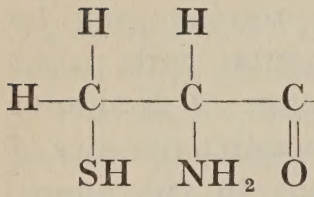
These facts are comprehensible only if the structure of the proteins is studied. Like the complex carbohydrates, the proteins may be said to be built up of fundamental units ; but in this case there is a wide range of dissimilar units to choose from, and the possible number of permutations and combinations in the aggregate—for the protein molecule is extremely large—almost beyond computation. The structural units of proteins are called *amino-acids* ; they are organic compounds with straight or branched chains or rings of carbon atoms (from two to eleven in number) ; one of the terminal carbon atoms enters into the formation of the acid " carboxyl " or —COOH group, and to its immediate neighbour is invariably attached the basic " amino " or $—NH_2$ group. The amino-acids are thus " amphoteric," that is simultaneously acids and bases, and this property is reflected also in the proteins. One or two amino-acid formulæ may be quoted :



$C_3H_7NO_2$, alanine.



$C_9H_{11}NO_3$, tyrosine.

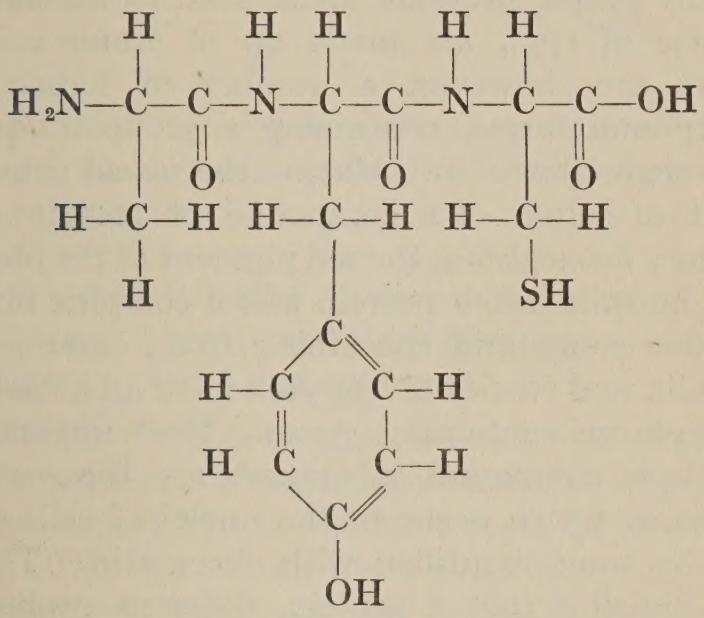


$C_3H_7NSO_2$, cysteine.

It will be noted that the last of these contains sulphur ; cysteine is, indeed, the most important sulphur-containing constituent of proteins.

The amino-acids are crystalline, colourless substances, soluble in water, in which they dissociate but slightly ; as we should expect from their " amphoteric " character, they combine with either acids or bases ; further, since the basic group balances the acid one, the solutions are approximately neutral. There are, however, exceptional forms which contain two carboxyl groups, and others which contain two amino groups, and these naturally are respectively acid and alkaline in solution. The most important property of the amino-acids, for the purposes of this discussion, is that they link themselves together, the amino group of one uniting with the carboxyl group of another, to form compounds called peptides—which are, we

have reason to believe, models of the more elaborate structure of the proteins. Thus the three amino-acids whose formulæ have been considered could unite to form a tri-peptide of the following constitution :

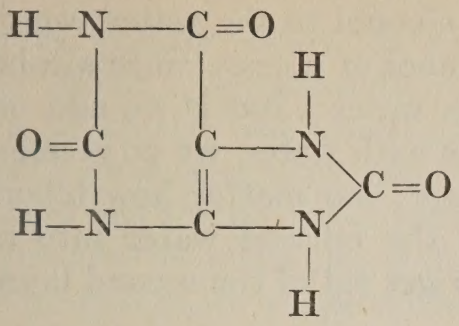


alanyl-tyrosinyl-cysteine, $C_{15}H_{25}N_3SO_8$.

It will be noticed that this peptide resembles the original amino-acids in containing one free carboxyl group balanced by one free amino group, and is therefore still amphoteric. The same is roughly true of the proteins, which we may now imagine as compounds similar to this tripeptide, but containing perhaps fifty or a hundred amino-acid molecules—not necessarily

all different, by any means. When we reflect that there are, however, at least twenty well-distributed amino-acids, we cease to wonder at the enormous number of distinguishable proteins occurring in nature.

The simple proteins, then, such as *ovalbumin* (white of egg), are made up of amino-acids; there are, however, a number of important compound forms, containing a group of quite different character. *Mucin*, the viscid constituent of saliva, is a compound of protein and sugar; *hæmoglobin*, the red pigment of the blood, can be split into a protein and a complex nitrogenous compound containing iron; *caseinogen* of milk and *vitellin* of egg-yolk have an attached phosphorus-containing group. Most important of these compound substances are the *nucleoproteins*, which occur in the nuclei of cells and are by some identified with chromatin. These are divisible into a protein, different probably for every species of living organism, and the complex *nucleic acid*, of which plants seem to have one type and animals another. Nucleic acid contains four molecules of phosphoric acid, four molecules of a sugar with six carbon atoms, and four molecules of four different purine bases. The purines are nitrogenous compounds, of which the best known (a product of the break-down of nucleic acid in the body) is *uric acid*



$C_5H_4N_4O_3$.

Nucleic acid and lecithin are the two most important phosphorus-containing constituents of living matter.

*The surface of contact between two phases is the locus of certain significant phenomena, chemical, physical, and electrical.*

It is not possible to understand the properties and importance of proteins without some grasp of the nature of the *colloidal* state of matter, and this in turn demands a brief study of the peculiar properties of the surfaces between phases. The meaning of the word " phase " is best brought out by example : thus, in considering a saucer full of water, we may take into account three *phases* : one liquid—the water ; one solid—the saucer ; one gaseous—the air. If a lump of sugar is thrown into the water, it constitutes a second solid phase, making four in all ; but when the sugar has completely dissolved, this phase has vanished, for a solution of sugar in water is but a single phase. If we

add a few drops of alcohol to the water, we do not increase the number of phases, since alcohol mixes perfectly with water; but if we add oil, which is not miscible with water, we do increase the number of phases; no matter how laboriously we churn up the oil and water into an emulsion, we cannot get rid of the second liquid phase.

The boundaries of phases have peculiar properties, of which the most elementary is that of *surface tension*, well seen at the interface between water and air. Here the surface tension acts as a force which strives to maintain the intactness of the surface and at the same time to reduce its area: it is surface tension which allows us—if we are dexterous enough—to float a needle in water as long as the surface remains unbroken; it is surface tension which causes water to rise into the capillary spaces of a lump of sugar; and it is surface tension which makes a soap-bubble or a raindrop spherical. A similar tension exists wherever two phases are in contact.

The surface tension of water, or any liquid, is considerably affected by dissolved substances. Inorganic salts may slightly increase the surface tension of water; inorganic acids and bases, and many organic substances such as sugars and amino-acids, have very little effect; but other organic compounds, notably alcohols and

esters, greatly *reduce* the surface tension, and are called "surface-active" bodies. Now it can be shown mathematically and it has been found experimentally, that if a dissolved substance has the power of lowering the tension at a surface, its molecules will tend to collect and congregate in the neighbourhood of that surface; though, as we know already, dissolved molecules usually distribute themselves evenly throughout the whole volume of the solution. Evidently the surface-active substances are partial exceptions to the general rule.

If we stir a little fine sand into water, and then drain or filter the water off, the sand left behind will not be by any means dry—so much we all know. Indeed, short of heating the sand or exposing it to dry air, it will be found very difficult to get rid of the last traces of the moisture, which forms a thin film around each grain, and is said to be "adsorbed" on the surface. Similarly, if water is allowed to trickle down a tube packed with dry sand, so that it finally oozes out at the bottom, we may wait for ever, but the last fraction of the water will never drip out at the foot of the tube; the weight of the water is not sufficient to drag away the adsorbed film from the sand-grains. If now—repeating a famous experiment—we pour through the sand a purple solution of potassium permanganate in water, the first few drops to trickle out will be *colourless*

—pure water with no dissolved permanganate. The explanation is that the permanganate molecules are even more powerfully adsorbed than the water molecules, and are thus left behind adhering to the sand grains; and the reason for this specially powerful adsorption is that the permanganate lowers the surface tension between the water and the sand, and therefore tends to accumulate where the two phases are in contact. If for the sand is substituted a more powerful adsorbent, such as powdered charcoal, and if the permanganate is replaced by a more surface-active substance, such as an organic dye, we may succeed in purifying the water almost completely in this way.

But there are other cases in which the concentration of molecules at the surface of a phase depends on quite different forces—on electric attraction. When a solid and a liquid are in contact, there is usually a difference in electric potential between them; tiny scraps of paper dropped on to water become charged with negative electricity, and the mutually repellent effect of these negative charges makes it difficult to bring the pieces of paper together. In the same way, if a tube full of water is loosely plugged with carborundum, the solid becomes negatively charged, the liquid positively; if an electric current is passed through the plugged tube, it will force the water to travel slowly

through the carborundum in the direction of the positive current. It is not hard to understand that this process will be much altered, if there is dissolved in the water an electrolyte—an acid, a base, or a salt, whose molecules dissociate into electrically-charged ions; for the negative charge on the solid will attract the positively-charged ions, till they become concentrated or adsorbed on the surface, while the negatively-charged ions remain in the bulk of the solution. In this way the difference of charge previously evident between solid and liquid is destroyed or masked, and an electric current no longer causes the water to move through the plug.

Different electrolytes differ considerably in their effect: the positively-charged hydrogen ion of acids is particularly potent, and so too are the positively-charged ions of calcium and some other metals, which carry twice or three times the normal unit charge of electricity. The molecule of calcium chloride, for instance, ($CaCl_2$) splits up into *three* ions: two negative ions of chlorine, each with a unit electric charge, and one ion of calcium, with a double charge of positive electricity. These doubly-charged ions are particularly active in destroying the difference of potential between two phases.

*In the colloidal state two phases are present in exceptionally intimate contact.*

It has already been hinted that some of the most important organic constituents of living cells do not truly dissolve in water, but form instead *colloidal* solutions or "sols"; and as we shall see in due time that protoplasm is a colloidal system itself, it is necessary now to examine the characteristics of the colloidal state. In a true solution the molecules of dissolved substance are independent of each other, coming into contact only momentarily and by chance; they are far too small to be made visible by any realizable process of magnification, too small to be held back by the meshes of a filter; the whole solution is uniform, and constitutes but a single *phase*. On the other hand, a suspension of the finest charcoal powder in water does not dissolve, and is, as we have seen, *two* phases—one solid and one liquid; where the two are in contact, there may be recognized the mechanical force of surface tension, as well as differences in electric potential. Each particle of the charcoal powder is made up of many molecules firmly bound together, and is large enough to be seen under the microscope or be held by a good filter. The molecules of a dissolved substance diffuse powerfully through the solution, so that an equal distribution is

maintained in all parts ; nor does their weight ever drag them to the bottom. But the charcoal particles diffuse slowly and feebly, and sooner or later yield to the attractive force of gravity and accumulate as a sediment at the bottom ; each particle carries the usual electric charge of a solid in contact with a liquid, but this is just not enough to protect the particle against the pull of gravity.

It seems very unsatisfactory, but nevertheless it is probably the safest plan, to define a colloidal solution as lying somewhere between these two extremes, of a true solution and a true suspension. The colloidal particles are larger than individual molecules, yet they pass easily through ordinary filter-paper ; but parchment retains them. They are too small to be actually *seen* under the microscope, but, unlike molecules, large enough to scatter rays of light, and can be seen as shining points in the " ultra-microscope " ; for the same reason, colloidal sols often appear faintly opalescent to the naked eye. The particles diffuse rather feebly, but they do not sink to the bottom like those of a suspension ; their electric charges are large enough to protect them against gravity. Truly dissolved molecules do not carry electric charges (except when they form ions), and therefore we say that the colloidal particles are large enough to be regarded as a second phase, very finely divided and in intimate contact with

the water, but none the less with all the properties of a surface between two phases clearly manifested.

The preceding paragraph may have suggested that colloidal properties are displayed only by very minute particles "dispersed" (to use a convenient word, without the implications of either "dissolved" or "suspended") in water, and this is so far wrong that it must be corrected. We must also introduce the word "micella" in place of "particle," which is a bad word since it suggests that the dispersed substance must be a solid. This is by no means the case; we can have colloidal droplets of oil dispersed in water, or of water dispersed in oil. The dispersion medium need not be a liquid at all: *mist* consists of colloidal droplets of water, dispersed in the air, *smoke* of solid colloidal particles in air. But in these pages we may practically confine ourselves to solid or liquid micellæ dispersed in water.

What, on the average, is the actual size of a micella? The diameter of a micella is a hundred times that of a small molecule; a hundred micellæ, joined end to end, would be about as long as some of the bacteria, which appear very small indeed under the microscope, and which are in fact about one ten-thousandth part of an inch in length. But these figures are very rough: there is much variation.

Finally, a more detailed discussion of the forces which prevent the micellæ from joining together, and eventually succumbing to the action of gravity, is required. The first of these is the electric charge on each micella ; electric charges of the same sign (positive or negative) repel each other, and make it difficult for two micellæ to come together, just as electric charges of the same kind make it difficult to bring together floating scraps of paper. But we know that these electric charges at the surface of contact of two phases can be destroyed by ions ; and we should expect to find, as we do find, that ions (especially those with a double or triple charge of electricity) destroy the charges on the micellæ and may cause them to run together and finally to sink. However, all the colloids in the living cell are protected in a second and quite different way ; they adsorb water molecules, which form a coating close around each micella ; so that if two of them should touch, there is not a real contact between their inner substances. This protective layer of water can be got rid of, nevertheless, if we introduce into the solution some substance which has an even greater affinity for water, alcohol for instance. But more important is the fact that many *ions*, such as the sodium ion, have a great affinity for water, and are indeed surrounded by water molecules attached to them in some way. Con-

sequently ions may not only rob the micellæ of their electric charges, they may also rob them of their sheath of water molecules, and by this double action destroy the stability of the colloid and cause the dispersed substance to become aggregated and precipitated. Even if matters do not go so far, it is easy to see that ions may greatly affect the degree of dispersion of a colloid.

*Living matter may be regarded as a colloidal solution, in which one phase is largely protein, the other a solution of inorganic salts in water.*

Although a few of the proteins, such as those of which silk and horn are composed, are very solid and insoluble, the majority can not merely be caused to form colloidal solutions in water, but will be found to occur naturally in this state. They do not form true solutions. Many students of the subject believe that the individual protein molecules are so large (having a molecular weight of 10,000 to 50,000) that they are already of the dimensions of colloidal micellæ; while others hold that the micellæ of even a protein sol are built up of many molecules.

The presence of colloidal protein in water has the effect of greatly increasing the *viscosity* of the fluid, so that it flows very sluggishly; concentrated sols may be quite treacly, or even set into a jelly. True solutions, on the other hand,

are never particularly viscous. But proteins have a very small *osmotic pressure* when compared with dissolved substances, because the micellæ diffuse so feebly and are relatively so few; accordingly, the *vapour pressure* of a protein sol is near to that of pure water. The *surface tension* of water, where it comes in contact with other phases, is greatly reduced by proteins, which must therefore be classed as surface-active substances; the micellæ obey the law which was stated for the molecules of surface-active substances, and tend to concentrate upon the surfaces where they lower the tension; this may go so far that a continuous membrane or "skin" of protein is formed. Everybody has seen this happen at the surface of warm milk.

The study of the electric charge on the micellæ, though unfortunately rather difficult, is of much interest in that it reveals the links between the chemical structure of the proteins and their colloidal behaviour, as well as explaining some of the most important properties of the proteins and of living matter in general. It is found that in an acid solution the protein particles are positively charged, while in alkali they are negatively charged. Now it will be recalled that the proteins inherit from their structural units, the amino-acids, the property of being "amphoteric"—that is, simultaneously or alternatively acids or bases. An acid is an electrolyte

whose positive ion is hydrogen, while the other ion varies from one acid to another. When a protein acts as an acid, the positive hydrogen ion or ions may be supposed to roam free in the water, while the whole great protein micella carries a negative charge, and may be regarded as a huge colloidal ion. On the other hand, when the protein is a base it fixes the hydrogen ions, and the colloidal ion is now positively charged, like the sodium ion in the base sodium hydroxide. If then the protein when dispersed in an acid is positively charged, it must be behaving as a base.

It has already been shown that acids and bases interact to form salts, the hydrogen ion of the former combining with hydroxide ions to form a molecule of water; hydrochloric acid and sodium hydroxide form sodium chloride (common salt) and water. The same process goes on even if the base is protein; it reacts with hydrochloric acid to form water and *protein chloride*—quite comparable to any ordinary salt, and well dissociated, though one ion is so enormously large. In the same way protein reacts with sodium hydroxide to form water and *sodium proteinate*—once again a true salt. In the first case the protein "ion" is positive, in the second negative. These salts with one large colloidal "ion" have certain peculiarities; for instance, the protein particle is unable to pass

through a membrane of parchment, ordinary ions and molecules can and do. If then a colloidal solution of a protein salt is enclosed in a parchment cup, and the whole immersed in water, the protein "ions" cannot escape, but the other ion, whatever it is, will diffuse a little into the outer fluid; there will then arise a difference of electric charge, inside and outside the membrane, since there are more ions of the one kind inside, more of the other kind outside. These electric-potential differences will also affect the distribution of other, purely inorganic salts that may be added to the solutions, disturbing their usual uniformity, and producing what is called the "Donnan equilibrium." It is likely that differences of potential of this type occur in living cells; but beyond this the point need not be laboured.

If protein particles are positively charged in acid solution and negatively charged in alkaline solution, there will clearly be some intermediate stage in the neighbourhood of neutrality at which they are not charged at all; this is called the "iso-electric point." It would no doubt fall exactly at the neutrality of pure water if the acid groups of the protein exactly balanced the basic groups, in number and in strength, but this is not so; it was observed before that some of the amino-acids of which proteins are composed are more acid than basic. The iso-

electric point of proteins usually lies somewhat to the acid side, and consequently proteins are most often negatively charged in neutral solution. At the iso-electric point, the particles, being no longer protected by electric charges, are least stable, and the protein is most easily precipitated. Let it be repeated that there still remains the protection of the thin layer of adsorbed water molecules.

Perhaps it is wrong to speak of these water molecules as a *layer*, since some of them are probably enclosed in the interior of the micella. In the case of a rather concentrated and very viscous, syrupy protein sol, let it be imagined that the micellæ begin to take up more and more water into themselves, so that the volume of the water outside decreases step by step. This happens, for instance, on cooling a colloidal sol of gelatine—a process familiar to every one. The dispersed micellæ become more and more crowded together; and finally, whereas they once swam freely in the fluid medium, they are now closely packed and separated only by a thin film of water, one from another. By this time the character of the solution as a whole has surprisingly changed: at first fluid, at a later stage very syrupy, it is now *solid*: a weak and tremulous solid, every one who has looked at a table-jelly will agree; but, nevertheless, a solid with the property of elasticity—which

liquids hardly show. The "sol" has been transformed into a "gel."

Of course, the process of removing water can be hastened by evaporation, so that finally a very tough and compact gel is produced, like india-rubber or dry gelatine. The latter (another familiar fact) will eagerly "imbibe" water and expand vigorously first to the tenuous solidity of a table-jelly, at length to the fluid condition. It probably happens that in some gels the solid phase, originally disperse, becomes continuous; and the liquid, once continuous as the dispersion medium, is distributed as minute droplets of colloidal size throughout the solid. This, however, is not typical: "gels" differ from "sols" in that the micellæ are very close together, separated by so thin a film of water that the whole is a coherent solid.

*The density of living matter is largely determined by the ions of the dissolved inorganic salts.*

The ions of inorganic salts affect the colloids in many ways, and especially do they affect the proteins—which, with the lipoids, are the most important colloids of protoplasm. The negative charge carried by the micellæ of most proteins in neutral solution is weakened or destroyed by the positively-charged ions of sodium, potassium, and calcium—the last most of all, since it carries a double charge on each ion.

But although each of these ions by itself has very much the same sort of action as its fellows, the combined effect of two ions acting together may be more than a little paradoxical. Thus, the sodium ions, and the calcium ions even more, tend to cause the micellæ of a sol to run together : they decrease the degree of dispersion if they do not actually produce a precipitate. But the combined action of sodium and calcium ions, in a mixture, is much weaker than that of either ion alone. It seems that though both seem to work in the same direction, when they work together they cancel each other out. The sol is much more stable in the presence of a mixture of salts than in the presence of a single salt. This phenomenon is known as the " antagonism " of ions, and crops up in so many ways in the behaviour and reactions of the living cell that one is glad to meet with it in the less complex domain of pure colloidal chemistry.

Then again, ions avidly attract the molecules of water, though in varying degree, and to some extent may be regarded as competing with the colloid micellæ for the ownership of water molecules. Since the transformation of " sol " into " gel " and back again is regulated by the entry of water molecules into the micellæ, very largely, it is not surprising to find it greatly influenced by the presence of ions. There is every reason to believe that both " sol " and

"gel" are represented in living protoplasm, and that there is indeed a constant alternation between the two phases. It has been shown how the fluid contents of the resting nucleus set to a semi-solid form when the cell is injured, or when cell-division is in prospect. The asters, too, must be regarded as temporary gels appearing in the cytoplasm. Small wonder, then, that the ions of the dissolved salts are considered to regulate to a large extent the physical state of the living matter. Slight alterations, possibly very local, in the concentration of these salts, or of their relative concentration with respect to one another (in view of the theory of "antagonism," this is plainly very important), may greatly affect the physical properties of the protoplasm and its different structural components.

We shall not be much misled, if we regard protoplasm as a colloidal system in which the dispersion medium is water, containing dissolved gases and organic substances, no doubt, but more significantly a delicately-balanced mixture of inorganic salts. On the other hand, the dispersed material must be very various physically and chemically. There are colloidal droplets of fat, and micellæ—we hardly know whether to consider them as solid or as liquid—of many different proteins, and of the lipoids, and of complex carbohydrates as well. In some parts

the system will be very fluid, in others so thoroughly gelatinous that it may be permissible to regard these regions as solid phases, and to ignore their colloidal structure. In any case, every stage of transition from the most liquid sol to relatively stiff gels is certain to be represented.

It is very plain that within the cell there is an enormous area of surface at which two phases are in contact—around each colloid particle or droplet, as well as around solid or liquid inclusions or at the external surface of the cell. We know that such surfaces are the seat of mechanical tension, of electric potentials, of unexpected concentrations of soluble or colloidal material; and we shall not be surprised to find in such surfaces the locus of intense chemical activity of every conceivable sort. It will, however, be unprofitable to attack this question until the nature of the reactions proceeding in living cells has been shortly outlined.

# CHAPTER III

## THE CHEMICAL DYNAMICS OF THE CELL

*Energy is set free when organic substances combine with oxygen.*

EXACTLY one hundred and fifty years ago (1777) the great pioneer chemist Lavoisier discovered the nature of the chemical reactions that go on when a candle burns, and demonstrated their essential similarity to the reactions from which the energy of living creatures is mainly derived. Candles are made of organic materials, whose molecules contain carbon and hydrogen; when the candle burns, these molecules are destroyed, and the atoms of carbon and of hydrogen unite with oxygen atoms from the air to make in the former case carbon dioxide ($CO_2$, carbonic acid gas) and in the latter case water ($H_2O$). It is possible to get pure, elemental carbon, in the form of charcoal, to "burn" and unite with oxygen to form carbon dioxide; and pure hydrogen gas also burns in air to form water. When water is formed, or when carbon dioxide is formed, a considerable amount of

energy is set free : in the burning of the candle, this takes the form of light and heat. It may therefore be said that the molecules of all organic substances, since they all contain carbon and hydrogen, are stores of *potential* energy, chemical energy ; when the molecule is oxidized or reacts with oxygen, this potential energy is set free as *kinetic* energy—most often as heat.

This is the most fundamental fact of biochemistry and of physiology, and immediately explains much. The living cells are machines which burn organic compounds to carbon dioxide and water, and convert the chemical energy of the more complex molecules into kinetic energy : the petrol engine and the steam engine are also, in the last examination, machines of this kind. Thus *eating* is necessary to life, because the cells must be constantly supplied with fuel, in the form of the complex organic molecules of the food-stuffs ; and *breathing* is no less essential, since the oxygen of the air must be brought into the body to combine with these molecules, or their energy cannot be set free ; and moreover, the carbon dioxide formed by the union of the oxygen with the carbon atoms must be driven out of the body. Every one knows that a man uses up the air in a closed room (or rather the oxygen of the air, since the nitrogen plays no part in these processes), and also pollutes it with the carbon dioxide he exhales ; a candle does

the same, more slowly; until at last, if there is no ventilation, the candle goes out, the man dies.

The living cells are, of course, specialized so that they oxidize only certain substances: a motor-car will not run on milk, and a man can derive no nourishment from coal or petrol. Moreover, the molecules of the substances which do enter into our daily bread are often too large and too complex to be oxidized directly by the cell, and require a preliminary splitting-up and simplification, in the process of *digestion*. It is, too, a matter of common knowledge that some foods—and, as far as that goes, some fuels—are better than others; some molecules yield more energy when oxidized than others, and this does not depend altogether on the amount of carbon and hydrogen these molecules respectively contain, but to some degree upon the structure of the molecule and the way in which the atoms are united within it. These questions will be considered again.

If an exception is made of the green plants, which actively entrap and utilize the energy that streams upon them in the rays of the sun, and also of some peculiar bacteria (simple plants) which derive energy from most extraordinary reactions such as the oxidation of sulphur or ammonia or iron, it may be said that all living creatures derive most of their energy from the

chemical energy set free when the carbon and hydrogen of the organic molecules of the foodstuffs combine with oxygen from the air to form, in the long run, carbon dioxide and energy. Living creatures " spend " the energy thus won in a variety of ways—firstly in the production of heat, to no small extent in the case of the warm-blooded animals ; secondly in active movement, muscular or other ; probably also in nervous activity ; and most certainly, they use the energy obtained from oxidation to carry out other chemical reactions, of a different nature. For while many chemical reactions *yield* energy, others *consume* energy, and will not go on unless energy is supplied to them ; it very often happens that in reactions in which large molecules are split into small ones, the trend is all downhill and energy is set free ; while the building-up of complex molecules from simple ones is nearly always an uphill task.

A candle burns, and in setting free energy forms carbon dioxide and water ; and it is not practicable, even if we have plenty of energy available to give back to the system in exchange for what it has lost, to rebuild the molecules of the tallow from carbon dioxide and water. The reaction is not reversible ; and this is generally true of the reactions in which organic compounds are oxidized, as in living cells (though it will be seen that the green plants do wonders

in this way). On the other hand, there are many chemical reactions which are reversible, and some of these are important to living organisms.

It is typical of reversible reactions that they are partly uphill and partly downhill. In the case of the reaction already mentioned, in which one molecule of the twelve-carbon-atom sugar called malt sugar or maltose reacts with a molecule of water and splits into two to form two molecules of the six-carbon-atom sugar glucose

$$C_{12}H_{22}O_{11} + H_2O = C_6H_{12}O_6 + C_6H_{12}O_6$$

this is quite well seen. A solution of maltose in water, under certain conditions, begins to react and glucose is formed; this goes on and on, and more and more glucose appears. No energy has to be supplied—this part of the reaction is downhill. But it is found that after some time there begins a reaction in the opposite direction: glucose molecules join together in pairs to form maltose molecules and water. Here again no energy is supplied to the system —this reaction is *also* downhill, apparently. As the one reaction waxes, the reverse reaction wanes, until at last they are both proceeding at the same rate: as fast as glucose molecules are formed, they join together; as fast as maltose molecules are formed, they split in two. In this way a balance, an *equilibrium* between the two reactions

is established and remains fixed ; the product of the reaction is a mixture of glucose and maltose in water. If the experiment had begun with glucose only, maltose would have been formed ; then the reaction would have set in in the opposite direction, and finally equilibrium would have been attained, just as before, and again a mixture of glucose and maltose would have been produced. The actual proportion of glucose to maltose depends largely on the concentration of the reacting substances, including the water. Since there must be a great excess of water present, the reaction in which water is used up is likely to be more powerful than that in which water is formed ; consequently, the "equilibrium mixture" will probably contain much more glucose than maltose. But if the amount of water is the same in each experiment, the equilibrium mixture will be exactly the same in composition, whether glucose or maltose was the starting-point (if all other conditions are kept constant). From the energy point of view, it is clear that from a pure maltose solution the way leads downhill ; from the pure glucose solution, also downhill ; and the bottom of the valley, to pursue the metaphor, is the state to which solutions of either glucose or maltose finally come—namely, the equilibrium mixture of the two. In a reversible reaction, energy is *set free* when the equilibrium mixture is formed

from either of the end-products. It must be admitted that the foregoing account takes no notice of many complicating features; the central facts are, however, sufficiently emphasized.

*The velocity of chemical reactions may be increased by high temperatures, by certain surfaces, and by certain specific substances.*

A candle burns quietly for hours, giving out energy as light and heat all the time, but it does not light itself; though the reaction is so plainly a "downhill" one, it has to be started with a lighted match. If a mixture of the gases hydrogen and oxygen is strongly heated, the two elements unite with explosive violence to form water, and much energy is liberated; but if the gases are perfectly pure and dry, it may prove impossible to get them to combine. At low temperatures a mixture of hydrogen and oxygen may be kept for years without any water being formed, but if a little powdered platinum is added combination takes place very swiftly and energetically, even explosively; yet at the end the platinum is the same as before, in no way chemically changed. A solution of pure maltose in water will be watched for a long time before any glucose appears in it; but if some hydrochloric acid is added the reaction travels quite quickly till the point of equilibrium is reached;

and yet at the end there is as much hydrochloric acid as there was at the beginning.

It is clear, then, that even a chemical reaction which liberates energy, which goes "downhill" as we have said, may proceed very, very slowly, quite imperceptibly; and that there are several means of hastening such a reaction. The first is by raising the temperature, as in lighting a candle; the second is by introducing into the mixture of reacting substances some special material, which speeds up the reaction without being used up itself; such a material is called a *catalyst*. The platinum is a catalyst which causes hydrogen and oxygen to unite at low temperatures, without being consumed itself; the hydrogen ions of the hydrochloric acid are catalysts which hasten the decomposition of maltose, and yet are not used up. It will be observed that platinum powder introduced into the mixture of gases constitutes a new *phase*, while the addition of hydrochloric acid to a solution does not; and it is very likely that there is a deep difference between the two cases.

The velocity of a chemical reaction depends on two main factors. In the first place the molecules which react together must first come together. In a mixture of two gases, there must obviously be innumerable contacts, every second, between molecules of different kinds, since all alike are in violent motion. The same is true of

the molecules of two liquids which truly mix, or of the molecules of two substances dissolved in the same liquid ; but in this case the number of contacts of molecules will depend on the concentration of the two dissolved substances in the solution. Ions will come together with special readiness, because of the attraction between opposite electric charges. On the other hand, the molecules of substances which are in different phases will have far fewer opportunities of reacting together, and such reactions will proceed slowly as a rule.

The second factor is more obscure but possibly more significant. It would seem that it is not enough merely for two molecules to come together for them to react, not even when the reaction is one which sets free much energy ; in many cases at least, the molecules must be in a certain state, an " active " state, or they will not affect each other. The physical conception of these " active " molecules is doubtful and difficult ; but the idea has proved so helpful in biochemistry that it cannot be entirely passed over. Perhaps it is best to regard an " active " molecule as one in a condition of *strain*, so that it readily breaks.

It is obvious enough that raising the temperature will accelerate a chemical reaction ; the molecules will move much more rapidly, there will be more collisions in a given time ; and

possibly heat also activates the molecules. But when we supply heat we supply energy, whereas adding a catalyst does not add any energy to the reacting mixture. From this it follows that a catalyst can never set an " uphill " or energy-consuming reaction in progress; rather is a catalyst to be likened to a lubricant, which allows a reaction, previously poised inertly on the slope, to slide downhill of its own weight. A block of iron may stand for ever on a sloping board, but if a little oil is applied slides easily downwards.

The peculiar properties of the surfaces between two phases have already been discussed; and where a catalyst forms an independent phase, it is natural to ascribe its action to some of these properties, and most of all to the *concentration* of molecules at the surface. It is also significant that such molecules concentrated at a surface are apparently not arranged in a higgledy-piggledy fashion, but may be packed together in a neat and orderly way (probably by electric forces), side by side and head to head like matches in a box. In agreement with this point of view is the fact that, on the whole, catalysts of this type are not very specific: powdered platinum catalyses a whole host of reactions, and in many of them can be replaced by various other powders, metallic or non-metallic. On the other hand, it is hard indeed

to understand the action of catalysts which are in the same phase as the reacting substances, as for example when hydrogen ions catalyse reactions of dissolved molecules. While it is true that there are many reactions which are accelerated by hydrogen ions, yet these are mainly of one definite type, and on the whole there is much specificity in catalysts of this kind: a reaction may be aided by one kind of molecule only, and that kind of molecule may not hasten any other reactions. There is no general theory of these "single-phase" catalysts; their mode of action is quite unknown, although of course it is always tempting to suppose that they enter into the reaction for a time, to form intermediate compounds which subsequently break down and liberate the catalyst in the same form as it had initially. This is plausible, but it is more than doubtful if it is always the real explanation.

The preceding paragraphs are undoubtedly rather difficult, and it may be well to summarize them in a sentence or two. Many chemical reactions, although they yield energy, proceed very slowly or do not proceed at all; but there are substances called catalysts, of quite different and unpredictable types for different reactions, which greatly accelerate these slow reactions, and yet at the end can be recovered in the same state and in the same amount as when they were added at the beginning. There are two

distinct classes of catalysts : those which constitute a separate phase, and those which do not.

*Organic catalysts of a peculiar type are of the very greatest importance in controlling the chemical reactions that go on in living cells.*

It is almost self-evident that living cells make much use of catalysts ; it could not be otherwise. For example, living cells oxidize, that is "burn," sugar to carbon dioxide and water—glucose if not cane-sugar ; but to oxidize sugar without a catalyst is by no means easy, and requires very considerable heat, although, once the reaction is started, energy is yielded by the oxidation. There are only two ways to start the oxidation of sugar to carbon dioxide and water ; one is to apply great heat, far above any that could be supported, much less produced, by living matter ; the other way is to make use of a catalyst, and if the chemist cannot find a suitable one, the living cell can.

Probably catalysts of both types are to be reckoned with. Thus the hydrogen ions of the very acid juice of the stomach act as catalysts in the process of digestion, accelerating the splitting of cane-sugar molecules into two halves, and so forth. On the other hand, the catalytic activity of the innumerable *surfaces* within the cell must certainly be taken into account. The evidence for this depends on two distinct lines

of research, both of which, especially in the work of Warburg, have yielded results of the highest importance, and will be referred to again. In the first place it has been shown that living cells lose much of their power to oxidize organic substances when their structure is destroyed by crushing or grinding with sand. It is plain that such violent treatment may have two effects: the first primary, namely the obliteration of the differentiated structures within the cell, such as the mitochondria and the nucleus, and the other secondary, the disturbance and destruction of the invisibly small surfaces of the colloidal micellæ of the living matter, which is so likely to be brought about by such rough handling; it was observed in the first chapter that the escape of the contents of the nucleus into the cytoplasm caused a general liquefaction therein, clearly a change in the degree of dispersion of the colloid constituents. It is not unlikely that considerable chemical changes also and inevitably accompany any disintegration of the physical structure of the living matter. More conclusive, perhaps, is the line of research which shows that chemical reactions which undoubtedly occur in living cells can similarly occur on non-living surfaces, such as the surface of charcoal in contact with solutions. The amino-acids, for example, when dissolved in water, resist oxidation with a considerable inertia; but if

powdered charcoal is present in the water, they are readily oxidized—a clear case of the catalytic power of surfaces. From analogy, then, it is argued that the surfaces in the living cell are aids to the oxidation of amino-acids, and no doubt other substances as well.

But there are to be found in all living cells catalysts of a peculiar and inimitable type, known as *enzymes*. There are enzymes to promote chemical reactions of every sort, and their importance can hardly be over-estimated; their properties must be carefully examined. The most singular character of enzymes is their great *specificity*; each of the many enzymes known catalyses either one single chemical reaction, or a group of very closely related reactions. Thus the three chief twelve-carbon-atom sugars, as was pointed out, all split up into two molecules of six-carbon-atom or hexose sugars; and all these three split very slowly in pure solution but quite rapidly in presence of the hydrogen ions of acids, which act as catalysts. These reactions can also be catalysed by enzymes, but there is no one enzyme which will catalyse all three reactions; for each reaction there is an enzyme. It was noted in the last chapter that the many hexose sugars are extraordinarily similar in their chemical structure; yet enzymes which attack one may be without action on another.

Enzymes do not act only *within* cells. Thus Buchner long ago ground up the cells of the microscopic yeast plant, and obtained a filtered juice which caused sugar to ferment just as the living yeast does. Moreover, there are many enzymes in the digestive juices, which are the products formed by living cells but not themselves alive. On the other hand, enzymes are not truly dissolved substances, but are undoubtedly colloids. On this fact depends a number of their properties; for instance, they are very sensitive to the acidity or alkalinity of their solutions, and many enzymes which are active in acid solution are totally inactive, perhaps completely and irreversibly destroyed, in alkaline solutions. Then again, enzymes are affected by temperature; there is a temperature at which they are most active, another (usually about 60° Centigrade, or the temperature at which water feels unpleasantly hot) at which they are totally destroyed. In many other ways, more directly, enzymes give evidence of their colloidal character.

The chemical nature of enzymes is unknown. After years of patient and difficult work, Willstätter has obtained very concentrated enzyme preparations, several thousand times more active than the juices obtained by crushing living cells; yet the secret is unsolved. He concludes, however, that the enzyme is divisible

into two closely-united parts: the first and larger a colloid, whose chemical nature is of secondary importance, but which confers on the enzyme many of its properties (sensitiveness to reaction and temperature, for instance); while the other portion, still most mysterious, is a rather small group of atoms with the power of accelerating a few *specific* chemical reactions. It is not unreasonable to suppose that the colloid exerts the common catalytic activity of surfaces, and produces local concentration in the solution; while the action of the unknown group is possibly to be compared with that of catalysts of the second type, those which in some still obscure way accelerate reactions without forming a separate phase. In any case enzymes display the usual property of catalysts: they increase the velocity of energy-yielding reactions otherwise slow or imperceptible, and are not used up or altered (or only transiently) in so doing.

Enzymes share with other catalysts of all sorts an interesting property, not hitherto discussed. Where the reaction they catalyse is a reversible one, like the splitting of the maltose molecule, they accelerate both sides of the reaction. Thus from either starting-point, maltose or glucose, the equilibrium mixture will be formed much more rapidly in the presence of the appropriate enzyme, or in the presence of

the hydrogen ions which likewise catalyse this reaction in both directions. So that this one enzyme may at one time, when pure maltose is the starting-point, promote the formation of glucose ; and at another time, when pure glucose is the starting-point, appear to promote the formation of maltose. What does happen is that the enzyme accelerates the formation of the equilibrium mixture, and does not alter the proportion of the two sugars in this mixture ; *that* can only be done by varying other conditions, such as the amount of water present, but never by adding a catalyst. The reactions which consume energy and will not go on unless energy is supplied to them cannot be set in train by a catalyst ; a catalyst is not a source of energy, it is merely a "lubricant."

*Enzymes play a large part in the simplification of the molecules of the food-stuffs during digestion.*

In all the higher animals the food-stuffs are considerably simplified before they are taken into the living cells. Thus the protein molecules of the food are attacked and split up by the digestive fluid poured into the stomach by the cells which line it ; this juice contains hydrochloric acid and an enzyme (pepsin) which attacks proteins ; further along the alimentary canal the acid is more than neutralized by alkaline sodium carbonate, and another protein-

splitting enzyme (trypsin) is contributed by a large gland called the pancreas ; the intestine provides yet another enzyme (erepsin), and at this stage the protein molecules are completely broken down into their constituent units, amino-acids, and these amino-acids are absorbed by the cells lining the small intestine and pass thence into the blood. The complex carbohydrates are also attacked at different stages in the passage of the food down the alimentary canal ; first by an enzyme in the saliva, then by the acid juice of the stomach, then by an enzyme (amylase) produced by the pancreas, until in the intestine only six-carbon-atom sugars remain, and these are absorbed and pass into the blood. Cellulose alone resists digestion and is unaffected by these enzymes, but it is destroyed very largely by the millions of bacteria which live and flourish in the large intestine. The fats are similarly, if only temporarily, split up during digestion, chiefly by an enzyme secreted by the pancreas (lipase ; its action is greatly aided by the bile formed in the liver and poured into the alimentary canal), and set free molecules of fatty acids and of glycerine ; these are absorbed, but combine again immediately as fats, and pass only rather indirectly and by a special channel to the blood stream.

All these reactions are of a definite type which has already been considered in some detail, in

the splitting of maltose to form two molecules of glucose :

$$C_{12}H_{22}O_{11} + H_2O = 2C_6H_{12}O_6.$$

A large molecule divides into two smaller molecules, equally or unequally, and at the same time a molecule of water is used up and its atoms distributed to the products of the reaction. Such reactions are called " hydrolytic," meaning " splitting by water," and all the chemical reactions of digestion are hydrolytic. It is typical of hydrolyses that while they are energy-yielding reactions (otherwise they would not take place in digestion), the amount of energy they yield is small. Consequently the total potential chemical energy, ready to be set free in oxidations, of a protein molecule is not very much greater than the total potential energy of the many molecules of amino-acids of all sorts which represent it. Although digestion, as a " downhill " process, can go on of itself in the presence of catalysts (enzymes) and without any supply of energy, yet there is not much loss of energy in the reactions as a whole. Hydrolytic reactions are always readily reversible, like the splitting of maltose ; but in natural digestion there is always a great excess of water present, so that the reverse reaction is negligible.

The simplified food-materials find their way into the blood-stream, and are carried thereby

to all parts of the body, and taken up here and there as required by the cells of the tissues. In particular the *liver* is the great clearing-house at which the absorbed molecules are sorted and stored or further altered. The fats are either rapidly consumed, or stored in various parts of the body; the sugars are consumed, or built up into the complex carbohydrate glycogen which is stored in the muscles and the liver. The amino-acids may be used to build up molecules of proteins, united not as they were originally, but in the pattern characteristic of the proteins of the body which now possessed them; or if they are not required for new growth or to replace wear and tear in this way, the amino-acids may, like the sugars and fats, serve as sources of energy. For this purpose they are freed of their $NH_2$ or amino groups, which form ammonia ($NH_3$); but as ammonia is a harmful substance, it is combined with carbon dioxide to form the waste product *urea*, $CH_4N_2O$, which is excreted from the body in the urine. The remainder of the amino-acid molecule now contains only carbon, hydrogen and oxygen and can be oxidized to carbon dioxide and water in the usual way, yielding energy.

Up to this point nothing has been said of the influence of the oxygen atoms in the molecules of food-stuffs. Petrol contains no oxygen at all, only carbon and hydrogen; for each atom

of carbon in the molecule, two atoms of oxygen from the air are required for complete combustion (to $CO_2$), and for each atom of hydrogen half an atom of oxygen from the air, to form water ($H_2O$). But the fats, the carbohydrates, and the amino-acids alike all contain oxygen, and must therefore be regarded as already partly oxidized. Thus the complete combustion of a molecule of glucose requires only twelve atoms of oxygen from the air, two to combine with each carbon atom ; there is already enough oxygen in the molecule to form water with the hydrogen atoms.

$$C_6H_{12}O_6 + 6O_2 = 6CO_2 + 6H_2O.$$

The fats and the proteins, on the other hand, contain rather less oxygen, and consequently require more oxygen from the air ; and as it is possible to measure the amount of oxygen taken up by an animal, it is possible to tell approximately whether carbohydrates, or fats, or proteins are being oxidized by the body, since each requires a different amount of oxygen. This is true not only of the higher but of all animals : measurement of the amounts of oxygen used up and the amounts of carbon dioxide exhaled are a valuable index to the chemical transformations, especially oxidations, going on in the living cells.

The amœba is a single cell, and has of course no alimentary canal. It engulfs the bacteria

upon which it largely feeds, surrounding them with its protoplasm ; it swallows them as a rolling drop of water swallows a grain of sugar in its path. The process of digestion, then, goes on in this case *within* the cell, and not, as in the higher animals generally, in a space lined by cells. But even in this simplest case the ingested bacterium lies, not in the cytoplasm directly, but in a little droplet or vacuole enclosed in the living matter. The changes which go on in this vacuole have been followed as closely as the minuteness of the object permits. It is at first acid, and this kills the bacterium ; then it becomes alkaline, and the bacterium dissolves slowly, and its substance passes into the protoplasm of the amœba ; the vacuole finally disappears. It is impossible not to be reminded of the digestion of proteins in man and the higher animals, which goes on first in an acid and then in an alkaline medium ; but it might be unsafe to insist on the parallel.

Digestion within cells is not confined to the single-celled animals, the Protozoa. It is met with as a normal thing, at least for some foods, in many of the simpler forms and even up to the snails. In the vertebrates generally, in man for instance, it plays no part in the normal digestion of the food. But there are cells in the human body which have the power of engulfing bacteria and other foreign bodies, and of digesting them

—the phagocytes of the blood and similar cells in various tissues; this is regarded as part of the body's system of defence against microbes. Yet apart from this, it must be considered that the processes of hydrolytic simplification of complex molecules and converse building-up of complex from simple molecules probably go on in all cells of the body; and it is not so surprising to find in most cells evidence of the presence of enzymes catalysing these hydrolytic and reverse reactions.

*Oxidation, the union of substances with oxygen, is the converse of reduction, the union of substances with hydrogen.*

Hydrogen gas is inflammable: that is to say, it unites eagerly, under suitable conditions explosively, with oxygen to form water. The hydrogen is oxidized, the oxygen is said to be reduced. When an organic compound combines with hydrogen it is similarly said to be reduced. Thus formaldehyde, $CH_2O$, can be oxidized to formic acid, $CH_2O_2$, or can be reduced to methyl alcohol, $CH_4O$; gaining in the one case an atom of oxygen, in the other two atoms of hydrogen. Now it is clear that if we compare the complete oxidation of these three substances to carbon dioxide and water, which takes place when they are burnt, they differ in the amount of oxygen from the air which they require; each molecule

of formic acid requires *one* atom of oxygen; each molecule of formaldehyde *two* atoms; and each molecule of methyl alcohol *three* atoms.

$$CH_2O_2 + O = CO_2 + H_2O$$
$$CH_2O + 2O = CO_2 + H_2O$$
$$CH_4O + 3O = CO_2 + 2H_2O$$

When we reduce formaldehyde to methyl alcohol, we increase its power of combining with oxygen; when we oxidize to formic acid, we decrease this power. From these considerations we arrive at the important idea that oxidation and reduction are opposite or converse processes.

To exemplify this rather further, let the reverse reactions to those described above be surveyed. Methyl alcohol can be converted into formaldehyde, $CH_4O$ to $CH_2O$; this decreases the power of the molecule as a whole to combine with oxygen, and it is therefore to be regarded as an oxidation, although the number of oxygen atoms in the molecule is not increased. Again, the conversion of formic acid into formaldehyde, $CH_2O_2$ to $CH_2O$, increases the molecule's power of combining with oxygen, and is therefore classed as a reduction, though the number of hydrogen atoms in the molecule remains the same. The conclusion is that oxidation is either an addition of oxygen or a removal of hydrogen from the organic molecule; reduction is either an addition of hydrogen or

a removal of oxygen. Here it may be noted, parenthetically, that in inorganic chemistry the terms oxidation and reduction have recently acquired a very wide chemical meaning, being applied to reactions in which neither hydrogen nor oxygen take part, and at the same time a restricted if rather uncertain physical meaning; but this does not require consideration in these pages.

The relation between oxidation and reduction becomes even clearer if the possibility is considered that an aldehyde may be simultaneously oxidized and reduced, some of the molecules forming the acid by oxidation, others forming the alcohol by reduction:

$$2CH_2O + H_2O = CH_2O_2 + CH_4O.$$

Here there are plainly two reactions, of which one, the oxidation, yields energy; but the other, the reduction, is an "uphill" reaction and requires a supply of energy. This supply of energy for the one reaction is clearly forthcoming, in the energy yielded by the other reaction. Without the oxidation the reduction could not go on; there would be no supply of energy to drive it "uphill." It will be seen that such interdependent, "coupled" reactions are of great importance in biochemistry; for the idea is readily extended to cases in which the molecule which is oxidized is not the same as

the molecule which is reduced. Here indeed we may say that hydrogen is transferred from the one molecule to the other, which " accepts " it and is reduced, or that oxygen is transferred from the one to the other.

This at last leads us to the most general case of oxidation and reduction, which may be stated in this way: when one substance is oxidized, another is reduced, and *vice versa*; this is true whether the oxidation is an addition of oxygen, a removal of hydrogen, or both. The three cases may be illustrated by the following equations, in which " XH " is the substance oxidized, and " YO " the oxidizing agent:

$$XH + YO = XHO + Y$$
$$XH + YO = X + YHO$$
$$XH + YO = XO + YH$$

In all these cases XH is oxidized, and YO reduced, whether hydrogen or oxygen is the substance transferred.

*Catalysts of several kinds promote oxidations in living cells.*

It may be as well to explain at the beginning that there are not a few substances which become partly or completely oxidized simply by contact with atmospheric oxygen, without high temperatures or the intervention of catalysts. This is called " autoxidation " and is by no means so

simple as might at first sight appear. The oxidations so far considered have always been "downhill" reactions yielding energy, and the formation of carbon dioxide and water has been called "complete oxidation." But every one knows that hydrogen forms another compound with oxygen, containing even more oxygen than water does: this is called hydrogen peroxide ($H_2O_2$). Why then is water regarded as a state of complete oxidation, if it is possible to add another oxygen atom to the molecule? The answer is that to get this second atom of oxygen in requires energy, and is an uphill reaction. It is a familiar fact that hydrogen peroxide decomposes readily, to form water and free oxygen. In the water molecule, the hydrogen may be regarded as quite saturated with oxygen; in the peroxide molecule it is more than saturated, it is overcharged with oxygen, and willing to get rid of the excess. It is believed that there are many organic molecules, more or less complex, which have the power of forming peroxides, that is of taking up an excess atom of oxygen, if energy is supplied to them and oxygen is present. It is further believed that when a compound oxidizes itself spontaneously, by autoxidation, it does so in two ways at once; two reactions go on, the first the formation of an ordinary compound of oxygen with the organic molecule, a reaction which yields energy, the second the

formation of a peroxide, a compound with extra or excess oxygen, whose formation is only possible because the first reaction supplies energy up to the requirements of the second. The two reactions are, in short, coupled. When a compound combines with oxygen spontaneously, it forms in part a peroxide, or compound containing an excess of oxygen readily given up.

We return now to the important experiments of Warburg, already mentioned, which showed that while amino-acids dissolved in water did not spontaneously oxidize, they could be caused to oxidize by the catalytic action of finely-divided charcoal. These experiments revealed certain interesting and informative facts which have not yet been touched on. It was found, for instance, that the accelerative action of the charcoal could be almost stopped by the addition to the solution of cyanides (very poisonous inorganic salts) or of organic substances of the class, chemically very various, known as *narcotics* from their paralyzing action on living cells. Now it is a property common to all the narcotics that they are very " surface-active " substances, that is, they powerfully lower the tension at the surfaces where water is in contact with another phase, as with charcoal. The surface-active substances concentrate at such surfaces, and are said to be " adsorbed " by the charcoal. The amino-acids are only slightly

surface-active, the narcotics very markedly; hence it is reasonable to suppose that while the amino-acids are somewhat adsorbed by charcoal, the narcotics are very much more strongly adsorbed, to such an extent indeed that the amino-acid molecules are driven away from the surface and displaced by the molecules of narcotic. As far as the amino-acid is concerned, in that case, the surface might as well not be there, and naturally no catalytic activity is to be detected. This theory, acceptable as it is, does not explain the action of the cyanides, which depends on another aspect of Warburg's work. He found that the action of the charcoal was greatly increased if salts of *iron* were added to the solution, or if the charcoal already contained iron in traces as an impurity (which it usually does). The conclusion was finally reached that the iron atoms, few and scattered as they were, were essential for the oxidation (or at least replaceable only by some other less important metals); the iron is supposed to bring the atoms of oxygen into the " active state " in which they combine readily with the amino-acids. The charcoal model thus reminds us of the enzymes, in which special activity is ascribed to a small unknown atomic group distributed here and there on the surface of the colloid, the iron atoms are distributed here and there on the surface of the charcoal, and exert

their own mysterious influence to hasten the oxidation of the amino-acid molecules, already concentrated to some degree in the neighbourhood by purely "surface" forces. The cyanide ions are supposed to combine with the iron atoms in some way, and rob them of their power to promote oxidations. Warburg believes that in the living cell, as in the charcoal model, the oxidations take place at surfaces where molecules are concentrated and by the immediate agency of iron atoms upon these surfaces. Cyanides are supposed on good evidence to kill cells by "paralysing" the iron atoms. Probably most investigators are disposed to agree that in living cells a very important part at least in the oxidations is played by iron atoms at surfaces; there is more doubt, however, in regard to the view that the iron owes its potency to an ability to "activate" oxygen atoms, for which the evidence seems rather weaker.

Biochemists of another group, headed by Wieland, believe that in the oxidation of organic substances by far the most important feature is a transfer, not of oxygen *into* the molecule, but of hydrogen out of the molecule to some other substance which "accepts" it. The catalyst is supposed to "activate" one or two of the hydrogen atoms *within* the organic molecule to be oxidized, so that these leave the molecule

and combine with any "hydrogen acceptor" which happens to be present. Of course by far the most important hydrogen acceptor is oxygen, which combines with the active hydrogen to form water (except that it may sometimes, as part of a coupled reaction, form hydrogen peroxide). The theory is best supported by the following significant fact, that if oxygen is absent, some other substance may be shown to be reduced by the transferred hydrogen which it has accepted; the organic dye *methylene blue* has been much used experimentally as a hydrogen acceptor. It will be seen that while Warburg lays emphasis on the activation of oxygen, Wieland emphasizes more the activation of hydrogen, but the two views do not seem radically incompatible or mutually exclusive. The "active hydrogen" theory fits in well with our knowledge of the *oxidizing enzymes* and *reducing enzymes* (in final analysis undistinguishable, since oxidation of one molecule means reduction of another), which may now be considered.

The simplest of the enzymes which catalyse oxidations and reductions is that called *catalase*, which accelerates the decomposition of hydrogen peroxide to water and oxygen; it is very widely distributed in living cells. This of course is a reaction promoted equally well by many inorganic substances, such as platinum powder; it is a reaction easy to set in progress. The

*peroxidases* also catalyse the decomposition of hydrogen peroxide, or of organic peroxides which may be formed by autoxidations; but in this case the oxygen liberated is said to be in the active state, so that it readily combines with organic substances and oxidizes them. There is also a group of oxidizing enzymes (*tyrosinase*) which convert the amino-acid tyrosine and related substances into the dark, insoluble pigments called *melanins*, familiar as the colouring-matters of the negro's skin, of many insects, of slugs, in fact very widely distributed in plants and animals alike.

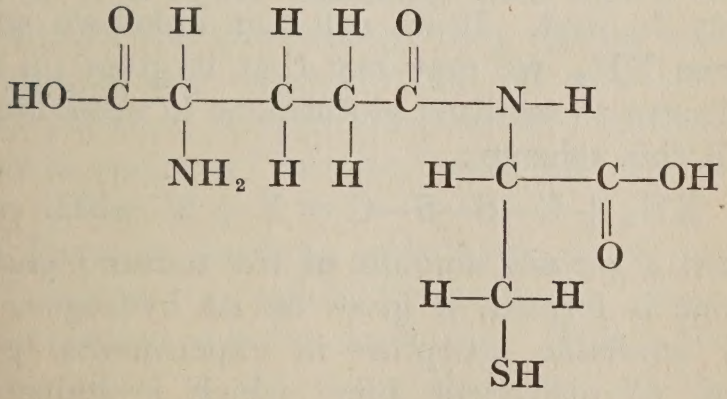
Better studied and more relevant to this discussion is the enzyme "xanthine-oxidase." This occurs, curiously enough, in milk as well as elsewhere, and promotes the oxidation of the purine base xanthine to uric acid:

$$C_5H_4N_4O_2 + O = C_5H_4N_4O_3.$$

Recent research suggests that in this case the mechanism of the reaction is as follows: the enzyme splits off two atoms of active hydrogen from the xanthine molecule, which then takes in the elements of a molecule of water ($H_2O$) to become uric acid. The two atoms of active hydrogen combine with dissolved oxygen, if any is present, to form hydrogen peroxide; if oxygen is not present, they may reduce any hydrogen acceptor available, such as methylene

blue, or nitrates (compounds containing the group $NO_3$) to nitrites (containing $NO_2$). The essential points here are, that although the reaction seems at a glance to consist merely in the entry of an atom of oxygen into the xanthine molecule, there is good evidence that the action of the enzyme is to remove hydrogen from the molecule as a preliminary; this clearly agrees with Wieland's views. Other known enzymes causing oxidations have been less studied, and need not be considered.

We turn now to the most important discovery by Hopkins of an organic substance, very widely distributed in living cells, which promotes oxidations catalytically, yet has a relatively simple chemical structure and is soluble in water. This substance, called "glutathione," is a compound of two amino-acids, glutaminic acid and cysteine, and has the formula



But our interest is centred mainly on the "SH"

or "sulfhydryl" group of the cysteine part of the molecule, and we may conveniently abbreviate the formula to G—SH. All such compounds, that is those containing the sulphur-plus-hydrogen or SH group, are very readily oxidized in a peculiar way: the hydrogen atom of the SH group is split off and two molecules then join together by their sulphur atoms:

$$2G—SH = G—S—S—G + 2H.$$

The particular importance of glutathione is that it actually occurs in living cells.

Glutathione, then, acts in living cells as a transporter of hydrogen, for the reaction given above is readily reversed. It seems that there is in the tissues a substance (not destroyed by heat any more than glutathione is, but, unlike glutathione, not soluble in water) whose chemical nature is not yet clear, but which certainly contains atoms of hydrogen with which it is ready to part. If we call this unknown substance $XH_2$, we may say that it gives up its hydrogen to oxidized glutathione in accordance with this scheme:

$$XH_2 + G—S—S—G = X + 2G—SH.$$

When a certain amount of the reduced glutathione is formed, it gives up its hydrogen, to any available acceptor—in experiments, perhaps, to methylene blue, which is reduced; more normally however to oxygen, with which

it probably forms hydrogen peroxide ; the excess oxygen of the hydrogen peroxide would then be available for combination with organic molecules, with the aid of the enzyme peroxidase. There is also evidence that glutathione itself forms an " organic " peroxide, and in this way may directly " transport " oxygen as it obviously transports hydrogen. These reactions of glutathione are deeply affected by the acidity or alkalinity of the solution ; and it is also found that they depend on the presence of small traces of iron, and are, in consequence, sensitive to cyanides.

The importance of glutathione to the cell is well attested by many experiments, some of which were made before there was any explanation to offer. For instance yeast cells or muscle cells removed from the body carry out oxidations and use up oxygen ; but if they are very thoroughly extracted with water, which removes the glutathione, they almost cease to consume any oxygen at all. If now the cells are provided with glutathione, they immediately begin again to " respire," and take up oxygen as before.

There is finally a substance, or a mixture of substances, called " cytochrome," also well distributed in living organisms. This is chemically allied to the non-protein part of the red pigment of the blood, hæmoglobin. It seems

that cytochrome may function as a transporter of oxygen in the cell, and promote oxidations in this way; and here, too, the presence of iron seems to be of importance. But the significance of cytochrome has not yet been thoroughly appraised, and it would be unprofitable to discuss it here at length.

The foregoing discussion has been difficult, and it will be well now to summarize the chief results. It was shown that there are certain organic molecules which spontaneously take up oxygen; but this process of "autoxidation" leads to the formation of peroxides, or compounds which have an excess of oxygen which they readily give up. It was then noted that charcoal containing iron presents to some extent a model of the living cell, in that it catalytically oxidizes amino-acids and some other substances unless narcotics or cyanides are present to interfere; on this is erected the theory of the rôle of iron as an activator of oxygen. This theory is countered by the theory that the splitting-off of active hydrogen from organic molecules is of fundamental importance, and this view is well supported by our knowledge of the best-studied of the oxidizing enzymes, xanthine oxidase. It may here be observed that there is no evidence that iron plays any part in the oxidation of xanthine by the enzyme, nor in some other oxidations where the activation of hydrogen is

very plainly the important feature. It is thus a tenable position to take up, that both theories are perfectly correct, and that when the activation of oxygen is called for iron must be present, but that when it is hydrogen alone that is activated the iron may be dispensed with. Thus washed muscle tissue oxidizes succinic acid by removing atoms of hydrogen from the molecule, so long as oxygen is present to "accept" the hydrogen set free. This reaction can be stopped by cyanides, which are presumed to "poison" the iron atoms at which the oxygen is activated; but if for oxygen another hydrogen acceptor, such as methylene blue, be substituted, cyanides no longer interfere with the removal of the hydrogen from the succinic acid.

Finally, it was demonstrated above that a great part is played in oxidations within living cells by substances which unlike enzymes resist heat, which do not depend for their action on surface forces, and which have a chemical structure not too complex to be unravelled; these are glutathione and the unknown reducing agent of the tissues, perhaps also cytochrome. It has been observed several times that hydrogen peroxide is formed in these oxidations, or on other occasions possibly organic peroxides; and here it is relevant to quote the conclusions of Dakin, that many peculiar substances introduced into the body are only partially oxidized,

and the products of this partial oxidation are the same as those experimentally obtainable by the action of hydrogen peroxide (and not of any other oxidizing agent) on the substances in question. Thus the animal body and pure hydrogen peroxide alike oxidize benzene to phenol (carbolic acid) and one or two other substances. It is thus more than likely that hydrogen peroxide is used by living cells to oxidize organic substances, with the aid of the enzyme peroxidase ; and we may perhaps regard the enzyme catalase, which decomposes hydrogen peroxide and is very widely distributed in cells, as a safeguard, getting rid of the peroxide should so much be formed that the structure of the cell itself is threatened with demolition by this powerful oxidizing agent.

*Phosphates play a part in the oxidation of glucose, which takes place in several stages.*

The amount of work which has been devoted to the study of the oxidation of so typical a food-stuff as glucose in the animal body is so enormous, and the results are so complex, especially since the influence of insulin on these reactions has become manifest, that it is impossible to present even a digest of it in these pages. We turn rather to the problem of the utilization of sugar as a source of energy by the simplest organisms, such as the microscopic one-celled yeast plant, in order to obtain some general ideas

on the subject. The action of yeast and kindred organisms upon sugar is known as "fermentation," and it proceeds in two quite different ways under different conditions. If oxygen is present, the products of the action of yeast upon sugar are as usual carbon dioxide and water; but if oxygen is absent, the yeast plant is still able to live and to transform sugar, though it cannot *grow*; from the sugar, without oxygen, are formed carbon dioxide and ethyl alcohol. It is upon this "alcoholic fermentation" by yeast that the making of beer and wine depends.

Glucose is a six-carbon-atom or hexose sugar, with the formula $C_6H_{12}O_6$, and one of the first steps in its destruction is that it is split into two. A glucose molecule halved gives two molecules of lactic acid, $C_3H_6O_3$. It seems likely, however, that a closely related substance, containing somewhat less hydrogen, pyruvic acid, $C_3H_4O_3$, is the first stage in the degradation of sugar by yeast. There are many "side reactions" at the next stage, such as those which lead to the formation of glycerine, $C_3H_8O_3$ (during the war German chemists found it practicable to obtain glycerine, a valuable constituent of certain explosives, from this source), but the main reaction is the splitting off of one carbon atom to form carbon dioxide, while the other two remain united, and pass by way of acetaldehyde, $CH_3.CHO$, to alcohol, $C_2H_5.OH$. It will be seen

that the total changes from glucose to carbon dioxide and alcohol,

$$C_6H_{12}O_6 = 2C_2H_6O + 2CO_2,$$

involve no oxidation or reduction, but consist in a mere simplification, a reaction which yields energy, though far less than that yielded by complete oxidation (every one knows that alcohol combines vigorously with oxygen, that is, it burns readily and hotly). Yet we have seen that oxidized or reduced products appear as intermediate stages or in parallel reactions, and these, one must assume, are mutually balanced against each other. It is evident that here there is a complex series of coupled and interdependent reactions.

The discoveries which were made twenty years ago by Harden and Young only add to the difficulty, but their general importance and wide application becomes more manifest every year. These workers found that fermentation proceeded much more rapidly in the presence of phosphates—such as sodium phosphate, $Na_2HPO_4$, an inorganic salt formed by the action of sodium hydroxide on phosphoric acid. The phosphate catalyses the reaction, for it is not used up. Subsequently, it was shown that there existed an actual compound between one molecule of sugar and two phosphate molecules, a *hexose-phosphate*. The only theory available

to explain the facts is that in its union with the phosphate the sugar molecule undergoes some slight change which makes it much more readily split in two parts by the action of the yeast; this change has been much discussed but remains obscure.

*The energy of muscular work is derived from the oxidation of carbohydrates, in a way which has been made strikingly clear.*

The discovery made twenty years ago by Fletcher and Hopkins that lactic acid is produced in the contraction of muscle fibres opened the door for a host of researches, in which the studies of Meyerhof on the chemical changes and of A. V. Hill on the energy changes are pre-eminent: these have now made it possible to give a very complete account of the chemical changes which take place when a muscle contracts. Muscle is, of course, a highly specialized tissue; but to some extent, surely, these results throw light on the chemical processes from which all cells, at least all animal cells, derive energy.

The muscle cells contain glycogen, the complex carbohydrate into which the six-carbon-atom sugars from the digested food are built for storage. When the muscle is stimulated to contract, normally by an impulse from the nervous system, the complex glycogen mole-

cules break up into fragments, and the ultimate fragments are molecules of lactic acid, $C_3H_6O_3$, each of which, as has been seen, is equivalent to half a molecule of glucose. Probably the lactic acid is formed directly from some intermediate substance, some "lactacidogen." In this process of simplification phosphates play a part, just as they do in alcoholic fermentation; some sort of *hexose-phosphate* is certainly one intermediate stage in the formation of lactic acid from glycogen. It will be noticed that these reactions involve neither oxidation nor reduction, but merely the splitting-up of a complex molecule into simpler ones. This process of simplification, nevertheless, sets free a considerable amount of energy.

Lactic acid, like any other acid, dissociates when it dissolves and sets free positively-charged ions of hydrogen. These hydrogen ions have an immediate and striking effect on the fibres of which the muscle is composed. A powerful tension is set up in the fibres, which strive to become shorter; it is this tension which causes the muscle to contract; and upon the contraction of muscle fibres depend all the active movements of our limbs, the beating of the heart, the process of breathing, and, in short, all the mechanism of motion in the body. There is no generally accepted theory to explain just *how* the hydrogen ions bring about this change

in the muscle fibres; surface tension, osmotic pressure, and all manner of forces have been invoked, but no agreement has yet been reached. The fact remains that the hydrogen ions cause the muscle fibres to contract with energy; or, if the ends of the fibres are so rigidly held that they are unable to become shorter, a tension is set up and the energy is dissipated immediately as heat.

The next stage in the process is that the lactic acid formed is neutralized; that is, it reacts with a base to form a salt, and the hydrogen ions go to form molecules of water and consequently cease to act on the muscle fibres, which thereupon relax to their original length. The neutralization is partly effected, perhaps, by dissolved inorganic bases, but no doubt very largely by the proteins of the muscle, directly or indirectly; the protein may be combined with inorganic bases (potassium) which are surrendered to the very much "stronger" lactic acid, so that a neutral salt is formed, the free protein being only very feebly acid. Here again, although there is still no oxidation, energy is set free. It must be carefully noted that the two reactions described, the formation and the neutralization of the lactic acid, although they both yield energy, are *reversible*; if sufficient energy is supplied to the system to "pay back" the amount set free in these reactions, it is possible for glycogen to be formed out of the neutralized lactic acid.

The only way in which a sufficient quantity of energy can be supplied is by an oxidation. If no oxygen is present, yet the muscle will contract and lactic acid be formed and neutralized, so that the fibres relax again and may be caused to contract and relax several times. Many tissues, and especially tumours, derive much energy from lactic acid formation. But if oxygen is present further changes take place, and the process of contraction becomes a much more economical one, so that the muscle will remain active and able to contract for a very much longer time in the presence of oxygen than in its absence. What happens is that a small portion of the lactic acid set free, about one-fifth of the whole, is oxidized completely to carbon dioxide and water. This yields so much energy that it is able to restore the muscle almost to the *status quo*. The total energy produced by the formation and subsequent neutralization of a given amount of lactic acid is only about one-tenth of the amount of energy yielded by the complete oxidation of this lactic acid. Even when, as in muscle, only one-fifth of the lactic acid produced is oxidized, so much energy is set free that only half of it is required to reverse the preceding reactions and convert the remaining four-fifths of the lactic acid back through hexose-phosphate into glycogen ; the other half of the energy of oxidation is dissipated as heat. Many

chemical problems, especially in regard to the phosphate, are still very obscure and may prove to be of great importance.

These, however, are the chief chemical reactions when a muscle fibre contracts ; of course in ordinary muscular movement there are complications, for there is a co-ordinated sequence of contractions and a maintenance of tension ; but the principle is unaltered. Consider the muscle fibre before and after a single contraction, such as that described above ; the only change is that a certain amount of glycogen has disappeared (since only four-fifths of the lactic acid can be rebuilt) and a corresponding amount of carbon dioxide has been formed. What is the reason for the apparently much more complex process that has been described, and why should a large amount of lactic acid be formed only to be converted again to glycogen ? The reason is not hard to find, since the energy and velocity of the contraction are clearly controlled by the amount of lactic acid produced (that is, by the concentration of hydrogen ions). In the apparently complex processes of simplification, neutralization, oxidation, and rebuilding, we have a beautiful and economical arrangement for producing the largest possible amount of lactic acid, temporarily, with the least possible permanent wastage of glycogen. In the absence of oxygen, since there can be no rebuilding,

muscular contraction involves a very great using-up of the stored carbohydrate of the tissue, and is wasteful; moreover, the accumulations of lactic acid are harmful, especially when they are so large that they can no longer be neutralized. It should be noticed that whereas the formation and neutralization of lactic acid, with the accompanying contraction and relaxation of the muscle, are very rapid processes, the oxidation of the lactic acid and rebuilding of glycogen are relatively slow. In continuous, violent muscular exercise the slower process is unable to keep up, so that lactic acid does accumulate. A man who has sprinted a hundred yards has much lactic acid stored in his muscles, and will go on breathing heavily and using up much oxygen for some time, until the whole of the accumulation is either (one-fifth) oxidized away or (four-fifths) rebuilt into glycogen; he is said to have "gone into debt for oxygen" during the time he was running, and consequently pants heavily as he repays the debt when the stress is over.

Let us now examine briefly the fate of the potential chemical energy released from organic molecules by their oxidation in living cells. It may, in the first place, appear directly as heat; in muscular contraction a large amount always does so directly, or the whole amount if the muscle is prevented from becoming shorter. Even if the muscle does contract, as in running

or the beating of the heart, it is only momentarily that the energy takes the form of mechanical motion; it is very rapidly converted into heat when it has served its purpose. We can, however, use our muscular energy to lift weights and place them on tables: in such a case some of the energy of the muscular movement has not appeared as heat, but is stored in the weight as physical potential energy; for the weight on the table has potential energy due to its position. When the weight falls off the table this potential energy is set free and appears once more and finally as kinetic energy in heat, and in noise which becomes heat as it dies away; though of course we may trap the energy of the falling weight in some way, by making it lift another weight, and so save the energy for a little longer. In general, however, the energy of oxidations in the cell appears sooner or later as heat. It may, temporarily, appear as electrical energy, for electric currents always accompany activity in living cells. Or again, energy of oxidation of one substance may be partly converted into potential chemical energy in the building up of another substance; as when half the energy of oxidation of lactic acid in muscle is spent in building up glycogen. Such processes of "synthesis" or building-up go on, no doubt, in all cells, and above all in the gland cells whose function it specifically is to elaborate special

substances, often of a complex character. No doubt the highest achievements of living cells, animal and plant cells alike, in the way of synthesis, are the enzymes : but we are so hopelessly in the dark about their structure that it is vain to pursue this question. Let us turn instead to the utilization of quite a different type of energy, the energy of the sunlight, by the green plants. The animals, as we have seen, derive almost all their energy from the simplification and oxidation of complex organic molecules ; without a supply of such molecules, in the food, they cannot exist. The same is true of the yeasts, which use up glucose much as animal cells do, and of most of the plants which have not the green pigment chlorophyll (the fungi in general). In the cells of the green plants there is also oxidation of organic molecules, with absorption of oxygen and exhalation of carbon dioxide—this in fact is characteristic of all living cells of whatsoever type ; but the green plants are able to build up these organic molecules for themselves, from carbon dioxide and water, by making use of the radiant energy of sunlight.

*Green plants are able to build up complex organic compounds from carbon dioxide by utilizing the energy of light.*

In all plant cells, probably, as in all animal cells certainly, complex organic compounds are

*simplified* and *oxidized*, typically to carbon dioxide and water, and in these reactions energy is set free. But in the *green* cells of plants, when light is available, processes in the opposite direction go on to a much greater extent: carbon dioxide is taken from the air and *reduced* and *built up* into complex organic compounds, and the energy which these reactions require is supplied by light. It is therefore possible for a green plant to grow and thrive, if light and carbon dioxide are available, on a solution containing only inorganic salts. These inorganic salts must include the elements necessary for living matter, such as potassium, calcium, iron, magnesium, nitrogen preferably in the form of nitrates, sulphur as sulphates, and phosphorus as phosphates. The air and the water supply the necessary carbon, hydrogen and oxygen.

The power of plants to assimilate carbon dioxide depends on the presence and properties of the green colouring matter, *chlorophyll*. In its chemical structure this substance is curiously similar to the red pigment of the blood, hæmoglobin, whose function it is to combine with oxygen in the lungs and to give it up to the cells of the tissues as they require it. It has been explained that hæmoglobin consists of a protein, united to a complex, nitrogen-containing group with which there is also bound up an atom of iron; when the protein and the iron are removed

the remainder is called a *porphyrin* ($C_{34}H_{38}N_4O_6$, for example). Chlorophyll consists of a very similar porphyrin group, united not to iron but to magnesium, and not to a protein but to an alcohol—a much simpler compound. $C_{55}H_{72}O_5N_4Mg$ is the formula of one type of chlorophyll; the chemical structure, like that of hæmoglobin, has not been fully worked out, but much is known about it, thanks largely to Willstätter.

In the cells the chlorophyll is concentrated in the *chloroplasts*, which are definite structures in the cytoplasm of the type known as plastids (see Chapter I). The plastids appear to have a structure similar to that of the rest of the protoplasm, that is to consist of colloidal proteins and lipoids, on which the pigments are apparently adsorbed. Characteristically, four pigments occur together: chlorophyll *a* and chlorophyll *b*, which are closely related; and two yellow pigments, carotene or carotin ($C_{40}H_{56}$), and xanthophyll. These two substances are chemically quite unlike chlorophyll, but nearly related to each other, and possibly to the lipoid cholesterol[1]; they occur as pigments in animals, as in butter and egg-yolk.

In the lower green plants every cell contains chloroplasts, but in the higher plants, as we know, the green pigment is restricted to certain parts and is most abundant in the leaves. It is clear that leaves, with their typically broad, flat

shape, are adapted to receive the largest possible amount of sunshine, and a further striving to this end is seen in the orderly arrangement of the leaves about the stem. Even in the leaf, however, every cell is not green; it is usual to find a layer of epidermal cells at the surface, which contain no chlorophyll and may indeed, as in the copper beech, contain other pigments altogether. This epidermis is pierced by an enormous number of tiny pores, called *stomata*, through which gases diffuse to and from the active, green cells in the interior of the leaf.

It is plain that the chloroplasts are the seat of the assimilation of carbon dioxide. If a leaf is freed from starch and then exposed to sunlight, but with one part screened off, starch forms in a short time over all the leaf except at the portion screened from the light. The presence of starch is readily detected by the intense blue colour which it gives with iodine. Microscopic examination shows in this way that the starch grains are actually formed by the chloroplasts, rather than by any other part of the cell.

The equation for the formation of starch from carbon dioxide and water can be written thus:

$$x\mathrm{CO_2} + y\mathrm{H_2O} + \text{light energy} = \mathrm{C}_x\mathrm{H}_{2y}\mathrm{O}_y + {}_x\mathrm{O_2}.$$

Just as oxygen is used up in the destruction of a carbohydrate, so must oxygen be evolved in the

formation of a carbohydrate. Green cells in the light, then, use up carbon dioxide and evolve oxygen much faster than they, with the other plant cells, use up oxygen and evolve carbon dioxide; although this latter process goes on in the dark as well as in the light, the green plants build up more complex carbon compounds than they break down; whereas the animals and the plants which are not green break down organic compounds, and do but little building up; they are, in fact, definitely unable to build up from carbon dioxide and water. A few exceptional bacteria will be considered later.

As the oxidation of starch or even glucose takes place in many stages, so we may expect that the formation of starch from carbon dioxide takes place step by step. Unfortunately we have almost no certain information about these steps, nor about the way in which the chlorophyll intervenes and the energy of the sunlight is brought to bear. Carbon dioxide dissolves in water to form carbonic acid, $H_2CO_3$ ($H_2O + CO_2$), and this is presumably the starting-point of the synthesis. It has very often been supposed that formaldehyde ($CH_2O$) or a hypothetical peroxide of formaldehyde ($CH_2O_3$) is the first formed product; but there is little real evidence that formaldehyde does occur in plants.

Willstätter and Stoll have suggested that chlorophyll first unites with carbonic acid (this

seems to be well established) ; that the carbonic acid group attached to the chlorophyll is in some way, with the aid of the energy of light, transformed into a peroxide of formaldehyde ; and that an enzyme then simultaneously liberates the excess oxygen from the peroxide and sets the formaldehyde free from the pigment ; and it seems fairly well agreed that from formaldehyde ($CH_2O$) simple sugars ($C_6H_{12}O_6$) may be formed simply by molecules joining together, with little consumption of energy. Baly and his colleagues have adopted a somewhat similar view ; they claim that ultra-violet light of a type not present in sunlight may form from a solution of carbon dioxide in water, without chlorophyll, a kind of formaldehyde which is particularly "active," so that it readily forms sugars or unites with inorganic nitrates to form organic nitrogen compounds ; they believe that chlorophyll may transform the light of the sun into the appropriate ultra-violet light ; and have erected a very elaborate scheme of the whole process, which finds little favour with most botanists. More acceptable, perhaps, is the theory of Warburg : he believes that chlorophyll is changed by light so that it gives off oxygen and becomes a very powerful reducing agent, which even tears the oxygen out of the carbon dioxide, or some simple compound thereof, so that the carbon dioxide or its modification is reduced and the

chlorophyll oxidized back to its original condition. There are many other theories, and it will be seen that we are far indeed from any real knowledge of the mechanism of carbon assimilation. The very careful work of Blackman and others on the conditions influencing the process, such as carbon dioxide concentration, temperature, supply of light, supply of water, and the like, is of high importance.

It may be well to refer here to those exceptional green plants which catch and digest living creatures, especially insects: the sundew is the best known. All these plants are able to build up complex carbon compounds with the aid of the sunlight, and as far as that goes the catching of insects must be regarded as a mere hobby, so to speak; possibly the compounds of nitrogen, or of other elements, derived from the insect prey are of considerable importance to the plant. The same applies to the parasitic green plants, like the dodder and the mistletoe, which form their own carbon compounds but draw upon other plants for supplies of the other elements required in the form of dissolved salts.

Of very great interest are the cases, by no means rare, of partnership between plant and animal cells. Thus the little flat-worm, *Convoluta roscoffensis*, beautifully studied by Keeble and Gamble, contains myriads of little single-celled green plants (algæ). These green cells

trap the energy of the sunlight, and build up carbon compounds to an extent sufficient to make their host fairly independent of other food. At the same time the animal body supplies the plant cells with carbon dioxide and with nitrogen compounds. There are many similar examples among the lower animals. Rather comparable, too, are the lichens, in which a partnership of the same sort has been established by a plant without chlorophyll (a fungus) and an alga or unicellular green plant. It should also be mentioned that there are one-celled organisms which behave in many ways like the simplest animals (Protozoa), but which possess chloroplasts like the algæ, so that it is hard to know where they should be classed.

*Some remarkable bacteria are able to assimilate carbon dioxide, making use of the energy derived from various and extraordinary reactions, instead of the energy of light.*

It has already been observed that there are organisms, certainly belonging to the plant kingdom, which derive energy solely from the oxidation or from the splitting-up of complex carbon compounds, as yeast does. Now while yeast, on the one hand, is a most adaptable creature, and will ferment very various substances, and survive (though fail to grow) in the absence of oxygen, there are many of its allies

which are less accommodating. Some demand oxygen, some cannot survive its presence and are called anaërobic ; many restrict themselves very narrowly to compounds of a particular type, which alone they are able to destroy. All fermentation and putrefaction is ascribable to the activities of such plants ; and it should be noted that while they live as the animals do, by destruction of organic substances, they often produce compounds quite different from the end-products of animal metabolism. Thus it has been shown that the animal body can do little with the nitrogen present in the food-stuffs, but is compelled to get rid of it in the form of ammonia ($NH_3$) or even of urea ($CON_2H_4$) ; the putrefactive bacteria, on the other hand, may liberate pure elemental, gaseous nitrogen ; or they may form pure hydrogen, or so unexpected a compound as methane (marsh gas, $CH_4$) which is oxidized by yet another group of organisms and yields much energy to them.

But there resides a peculiar interest in certain forms which are able to imitate the green plant in that from carbon dioxide and water they build up the complex organic molecules of which their living protoplasm is necessarily composed and yet are not green, and live very often in darkness. They do not use the energy of light to effect the reduction of carbon dioxide and the synthesis of complex organic compounds, but

utilize instead energy set free in other reactions often of a most surprising kind.

The sulphur bacteria, of which *Beggiatoa* is an example, live in waters which contain a little sulphuretted hydrogen ($H_2S$)—a gas whose evil smell is familiar to those who have had the misfortune to open a bad egg. This gas often accompanies volcanic disturbances, and is to be found in the waters of not a few mineral springs ; it is also produced by certain putrefactive bacteria from the sulphur of proteins and other compounds. *Beggiatoa* collects the sulphuretted hydrogen, and oxidizes it to free sulphur, while the hydrogen split off is " accepted " by oxygen and forms water or peroxide. The cells can be seen to be filled with granules of yellow sulphur, but the process does not stop there ; the sulphur is oxidized thoroughly to sulphuric acid ($H_2SO_4$). From these reactions the bacterium derives enough energy to assimilate carbon dioxide and convert it, by means quite unknown, into complex organic substances. The so-called " purple bacteria " carry out similar reactions, but are less well understood. Another group, rather a mixed one, carries out the oxidation of iron and uses the energy so obtained for carbon assimilation in the same way ; these forms are not uncommon, and become so clogged with particles of rusty iron hydroxide that they may give very serious trouble to water-works engineers. Yet

another group derives energy similarly from the oxidation of pure hydrogen to water, and may obtain the oxygen necessary for this reaction, not from the air but from compounds ordinarily regarded as very stable, such as nitrates (with $NO_3$ group) and sulphates (with $SO_4$ group), setting free nitrogen or sulphur.

Of greater practical importance are the bacteria which derive the necessary energy for carbon reduction from the oxidation of ammonia ($NH_3$) to nitrites (such as $NaNO_2$) and nitrates ($NaNO_3$). For we have seen that animals produce ammonia, or compounds from which putrefactive organisms set free ammonia, as they do also from plant debris; and the majority of plants prefer to obtain their nitrogen in the form of nitrates, though many can, at a pinch at least, make some use of ammonia. Along with these are to be classed, finally, the "nitrogen-fixers." We know that nitrogen gas constitutes four-fifths of the atmosphere, but it is a very inert element, and neither animals nor the higher plants are able to get the free element to enter into chemical combination. This is done, however, by certain bacteria, of which some (*Clostridium* and *Azotobacter*) live in the soil, and others live in the root-hairs of plants of one particular order, the Leguminosæ—peas, beans, furze, vetches, and the like; the plant gives carbon compounds to the bacteria, and in ex-

change receives compounds of nitrogen, in the making of which the nitrogen has been obtained from the air. The chemistry of the process is unknown, but its importance is tremendous, since, as has long been known, the richness of an impoverished soil may be restored (as far as nitrogen compounds are concerned) by allowing a leguminous crop to grow upon it. It is only in the last few years that the electric furnace has enabled chemists to " fix " atmospheric nitrogen, and made the farmer, potentially at least, independent of the nitrogen-fixing bacteria.

It would now be possible to write " the adventures of a nitrogen atom " as Addison wrote his " Adventures of a Shilling." We might imagine a nitrogen atom, at one time forming part of a nitrogen molecule in the air, captured by one of these bacteria and handed over to the host-plant, to be built up into a vegetable protein. This in turn might pass into the body of a herbivorous animal, the protein be digested to amino-acids, from one of which " our " nitrogen atom would be split off as part of an ammonia, and presently of a urea molecule. But before that it might have passed over to a carnivorous animal, and here possibly built up into some animal protein, not to be cast out from the body till much later. The urea or whatever compound our atom enters into is ultimately excreted and attacked by fermenting bacteria,

which again split off ammonia. The ammonia is seized by other bacteria, of which one first oxidizes it to a nitrite (*Nitrosomonas*), the next oxidizes the nitrite to nitrate (*Nitrobacter*). The nitrate molecule stands a good chance of being used by a plant, but might fall into the hands of one of those bacteria which steal oxygen from nitrates, to combine it with hydrogen ; in which case our nitrogen atom would be set free in the atmosphere once more, and the cycle would be complete. If our knowledge were more complete it would be easy to construct similar imaginary " life-histories " of other atoms, no less instructive and illustrative of the interdependence of all forms of life.

## CHAPTER IV

### THE PHYSICAL DYNAMICS OF THE CELL

*The living protoplasm is enclosed by a delicate membrane, through which some substances can pass readily, others only with difficulty.*

It is now generally agreed that the external boundary of living protoplasm is formed by a delicate membrane, which indeed is too thin to be seen under the microscope, but whose existence is indicated by a large number of experimental facts. In the first place, the protoplasm itself is usually far too liquid to have any coherence which could prevent the colloidal micellæ from merely scattering in water (and almost all cells are bathed either with water or with an aqueous fluid, such as the lymph of our own tissues—a fact which must never be lost sight of), were they not held in place by some stiffer envelope. The same applies, perhaps to a smaller extent, to the organic and inorganic compounds in the cell which are truly soluble in water; for these might be kept in place by being adsorbed on the surfaces of the colloids.

Yet these surface forces are inadequate to explain the enormous differences which may exist between the inorganic composition of the fluid inside the cell and that of the external medium. For example, the alga *Nitella*, a very simple plant, lives in pond water containing only five parts of sodium per million, and no potassium that can be detected; inside the cell, on the other hand, there may be 200 parts of sodium and 2,000 parts of potassium per million! Muscle cells contain potassium and no sodium, the lymph which bathes them more sodium than potassium. A tadpole may be placed in a salt solution of higher concentration than that which is found within the cells; but the salt molecules from outside do not make their way into the cells. This is not explicable unless there is a membrane at the boundary of the cell through which the salts are unable to pass. One more and very decisive experiment may be cited: a cell may be immersed in a solution of a dye, without any colour passing into the living matter; but if the dye is injected under the surface of the cell, directly into the interior, it spreads rapidly through the whole cytoplasm.

On the other hand, it is obvious that the external membrane must allow some substances to pass through fairly easily. In all cells there is a constant exchange of gases, oxygen and carbon dioxide, and of water also, between the

interior and the exterior. The larger molecules of the food-stuffs, glucose for instance, must be able to enter the cell at certain times at least, and similarly the waste products must escape from the cell. For the moment, then, we may regard the " plasma membrane " as a thin film through which some molecules, notably those of water and of gases, can pass readily, while the molecules or ions of salts pass slowly or not at all.

The development of the technique of micro-dissection, especially by Chambers, has made possible interesting experiments on the tearing open of plasma membranes. If this is done sharply at a particular point, not only is the membrane ruptured, but it falls apart completely. A breakdown of the structure of the film takes place, starting at the injured point, but travelling thence right round the cell, which presently falls to pieces. On the other hand, not only are these membranes fairly elastic, but the cell has a great power of rebuilding them very rapidly, under suitable conditions. If the tearing be done gradually, the cell may have time to save itself from destruction by forming a new surface film across the break, through which only a little of the contents has been able to escape. Some cells have this power so highly developed that if they are crushed or torn to pieces each of the drops of protoplasm set free

rounds up and surrounds itself with a membrane. It quite often happens to an amœba creeping over a rough surface that part of the cytoplasm sticks, is torn off, and gets left behind; but the tear in the plasma membrane is at once repaired, not merely by the amœba itself, but also by the marooned blob of protoplasm, which may survive for some time. In the process of cell-division in animals, a typical membrane develops on the new surfaces as soon as they appear.

In the discussion of the properties of solutions (page 35), some attention was given to membranes which allow the molecules of water to pass through, but retain the molecules of some dissolved substances, such as the ions of sodium chloride. It was seen that if a vessel containing a strong solution and lined with a membrane of this sort is immersed in pure water or a weaker solution, water passes into the vessel from the exterior until a considerable "osmotic" pressure is set up. Since the external protoplasmic film is apparently to be regarded as a "semi-permeable" membrane of this type, it is clear that osmotic forces may play an important part in the life of the cell. Consider for example red blood corpuscles (minute cells filled with the pigment hæmoglobin) experimentally removed from the fluid blood plasma in which they are normally suspended, and bathed instead in pure water. Inside the cell there is a fairly concen-

trated solution of inorganic as well as organic compounds; outside the cell is pure water; between there is a semi-permeable membrane, elastic but delicate. The result of the experiment can be foretold; water will pass into the interior of the cell, the internal pressure will rise; the membrane will stretch to the uttermost, but at last and inevitably burst, so that the soluble contents of the cell escape and are scattered. This is called "cytolysis" (or, in the particular case of the blood corpuscles, "hæmolysis".)

On the other hand, if the cells are immersed in a solution of high concentration, the reverse process will take place; water will leave the cell and be drawn into the more concentrated external solution; the cell will shrink. This is best seen in the case of many plant cells, in which the living protoplasm with its external film occupies a fairly rigid box-like chamber of cellulose and similar materials. If such cells are treated with a strong solution, water passes out of the living matter, which ultimately shrinks away from the encasing cellulose walls; this is called "plasmolysis." The cellulose walls act as a support, enabling the cells to withstand a very high internal pressure and preventing bursting; but they serve to reveal very clearly any reduction in the volume of the living matter. Cells vary greatly, not only in

their natural internal concentration of dissolved substances, but also in their ability to resist treatment with solutions either too weak or too strong; and the resistance of the cell is affected by many factors. Some plant cells are so sensitive, so easily plasmolysed by solutions only slightly too strong, that they are a reliable index of the molecular concentration of a solution; the theory of the dissociation of electrolyte molecules was supported, in its early days, by such experiments.

There are interesting points in the osmotic properties of animals which ordinarily live in fresh or salt water. In the case of marine animals, it is found that the simpler forms, including all the invertebrates and the lower vertebrates up to fishes, but not including bony fishes, have an internal concentration very close to the external concentration of the sea water in which they live. This does not mean that the composition of the internal and external fluids is the same, for it most certainly is not; a part of the internal osmotic pressure is due to organic compounds, and in any case the mixture of salts present in living matter is not the same as that in sea water (it has been suggested that the *proportion* of the various salts in living cells, which is rather constant, is that of the sea water of the very remote geological past). All that can be said is, that the number of dissolved

molecules, counting each ion as a molecule, in a given volume of the protoplasm or the internal fluids such as the blood is the same as in the same volume of sea water. Moreover, these lower marine animals are directly influenced by the osmotic pressure of the external medium; the internal pressure changes to suit the external. If a marine invertebrate is brought into dilute sea water, it takes up water and gains in weight until it has diluted down its internal concentration to that of the medium, or dies in the attempt. In concentrated sea water it will lose weight by losing water from its tissues until the concentration in the internal fluids again balances that of the medium. On the other hand the bony fishes and the marine reptiles and mammals do not change in this way to suit their environment; the osmotic pressure of their internal fluids is constant, and always very much less than that of sea water (about one-third as great). It is plain that, no matter how they may protect themselves by the development of impermeable scales and so forth, these forms must constantly lose water from their tissues to the more concentrated external medium; and they must, presumably, correct this by actively drawing in water to make good the loss. Other cases in which cells actively draw in water and even dissolved substances will come up for consideration later on.

Animals which live in fresh water are in very different case. It is true that they have, vertebrates and invertebrates alike, a low internal concentration of dissolved molecules; but they cannot possibly attain osmotic equilibrium with their medium, which may after all be practically pure water. Inevitably, then, water from without passes through the semi-permeable membranes of the body surface into the tissues, and has to be got rid of by being actively pumped out, along with the ordinary waste products of the cells' activities. It follows that all fresh-water animals and the higher marine vertebrates must have developed mechanisms, which constantly consume a certain amount of energy, for maintaining the concentration of their internal fluids at a fixed point against the disturbing influence of osmotic forces. The rhythmically pulsating " contractile vacuole " of many of the Protozoa is often, and reasonably, supposed to be chiefly occupied in draining the protoplasm of the excess water which continually finds its way in through the plasma membrane.

The property of semi-permeability may be examined still further in the case of dead cells. It is found that if a dead plant cell is immersed in a solution of an appropriate dye, which is exactly " isotonic," that is of the same molecular concentration as the internal solution, the dye does not penetrate into the cell. But if there is

any osmotic difference, the membrane is found to have lost its elasticity and power of being repaired ; it breaks or bursts and the dye passes freely into the dead cytoplasm. A very ingenious method of producing slight local injury in plasma membranes was found by Tchahotine. Using, as so many workers on the cell have done, the egg-cell of the sea-urchin, he directed a fine beam of ultra-violet light on one part of the surface. This point was somewhat injured by the light, and when now the cell was brought into a dilute solution, from which water was taken up and an internal pressure produced, the weakened spot on the surface film bulged out like a blister ; in a concentrated solution it formed a dimple, as the egg strove to shrink.

It has sometimes been argued that inorganic salts within the cell may not exert their full osmotic pressure, as they may be bound in some way to the colloidal constituents ; but there is now good reason to believe that, for the most part, they are not so bound. On the other hand, osmotic forces do not account for all the changes of pressure in living cells ; the " imbibition pressure " or avidity with which some colloidal gels take up water varies in different solutions, and is probably not negligible in the physical dynamics of cells ; but so little is known of this factor that it must be disregarded here.

*The plasma membrane is readily permeated by some dissolved substances.*

Hitherto the plasma membrane has been treated as if it were entirely semi-permeable, allowing only water to pass through, and keeping back all dissolved substances. But it is clear that at times at least it must be permeable to gases, to organic food-stuffs, and to organic waste-products, and it will be seen that it is also partly permeable to salts; it only approximates to the "ideal" condition, and the same is true of even the much more stable artificial membranes of copper ferrocyanide. It is plainly of much importance that the resistance of the membrane to all sorts of compounds, organic and inorganic, should be accurately known; since there is hardly a single physiological problem into which the question of cellular permeability does not enter. The absorption of digested food-stuffs by the cells of the intestine, their passage thence into the blood, their entry into the cells of the tissues where they are consumed; the escape of organic waste-products such as urea and uric acid from the tissue-cells into the blood, and their passage from the blood through the cells of the kidneys; the diffusion of gases through the walls of the lung; the escape of digestive juices and other secretions of all sorts from the gland cells; the

transmission of the nervous impulse from one cell to another; the response of cells to external stimuli; all these depend on the nature and properties of the plasma membranes.

Many methods have been used for testing permeability. It has been explained above that the plasmolysis of the plant cell depends on the inability of dissolved salts to pass through the plasma membrane; and when it is found that cane-sugar solutions of the requisite strength also produce plasmolysis, it is justifiable to assume an impermeability to cane-sugar molecules; when on the other hand it is found to be very hard to get cells to plasmolyse in strong solutions of urea, it is no less reasonable to suppose that the urea molecules can pass into the cell without too much difficulty, so that the external and internal concentrations are brought to approximate equality, and osmotic differences are slight. Similar methods can be applied to the study of hæmolysis, since this also depends largely on osmotic pressure.

A very satisfactory means of determining the permeability of a cell to various substances is available in the case of certain simple plants, *Nitella* and *Valonia*, in which are found enormous "vacuoles" or spaces filled with aqueous solutions, entirely surrounded by living matter. From *Valonia* vacuoles as much as five cubic centimetres of fluid can be obtained, and it is

easy to determine by chemical means the presence of any unusual substance which has been brought into contact with the outer surface of the cells and has penetrated right through into the vacuole; for it is assumed that the vacuole is lined by a membrane very similar to that which forms the external boundary of the cell. Chemical analysis can be of use in other cases, but seldom is it at once so simple and so decisive as in these examples.

Permeability may also be made manifest by changes which can be seen under the microscope being brought about when the cells are bathed in some particular solution. Most plant cells contain tannin compounds, which form an ink with any iron salts which succeed in entering the cytoplasm. Or again, cells are found which contain natural colouring matters that are "indicators," that is, they change colour when brought from acidity to alkalinity, or *vice versa*, or it may be possible to stain the cell artificially with a dye of this sort, such as "neutral red;" in either case, the penetration of acids or bases into the cell will be easy to follow. The penetration of a coloured substance, of course, may easily be followed by the eye. There remain some methods quite different in principle, for example those in which the permeability to electrically-charged ions is tested by following the resistance of a tissue to the passage of an

electric current; or those in which permeability factors are deduced from the behaviour of a whole organ or organism in a certain solution. All these methods, and many more, have been used to illuminate this fundamental problem.

When, in the last years of last century, Meyer and Overton and others made really extensive determinations of the power of different substances to penetrate into living cells, the results obtained were a little surprising. Cells proved to be very permeable to organic substances of a type very rarely met with in nature, and seldom of any significance to living creatures. These permeating substances included the ethers; the esters; the hydrocarbons, such as paraffins and benzene, containing only carbon and hydrogen; the alcohols, particularly those with long chains of carbon atoms and few hydroxyl groups; and a few nitrogen compounds. All these substances were either known before or then found to possess *narcotic* properties, depressing the activity of cells even in small doses; they were also all "surface-active" substances, some very markedly, but the importance of this was not recognized at the time; and they were all much more soluble in the "organic solvents," such as ether or benzene, than in water. On the other hand, compounds with many hydroxyl (OH) groups, such as glycerine and the sugars, or many carboxyl (COOH) groups and short

chains of carbon atoms, like acetic acid, or with amino ($NH_2$) groups like the amino-acids, are less surface-active, are soluble in water rather than organic solvents, are not narcotic, and penetrate very slowly into living cells, although in this group are included all the organic compounds which one can with certainty affirm do in nature pass in and out of living cells in the course of their ordinary life—namely, the foodstuffs and the usual waste-products.

From the fact that so many of the compounds which penetrate quickly into cells are soluble in the organic solvents, Overton deduced that the living cells were surrounded by a layer of some substance which had the properties of an organic solvent; and of all the constituents of living matter those that seemed best fitted for this rôle were the lipoids, of which lecithin and cholesterol are the most important. Substances which dissolved in lipoids very readily were able to pass through the membrane without difficulty; but they altered the nature of the membrane to such an extent that they acted as narcotics on the cell. Substances insoluble in lipoids were unable to pass into the cell, unless by some extraordinary and unexplained mechanism. Overton's theory had many faults; but it explained much, and in a more or less modified form has formed the basis of most theories of the cell membrane since that time. From these

theories one has been selected for the purposes of this book, since it seems to the author that it is the simplest and accords best with the known properties of the cell membrane in particular, and of semi-permeable membranes in general.

*The plasma membrane is a thin film of a colloidal gel.*

The plasma membrane of living cells is a thin, coherent film of a colloidal gel—a structure probably common to all membranes. The nature of gels has already been discussed (page 70), but may appropriately be recalled here with special reference to the point at issue. It was seen that a gel differs in degree rather than in kind from the more typical form of colloidal dispersion, the sol. In a colloidal sol of gelatine, for example, the gelatine is distributed through the dispersion medium—water or an aqueous solution—in micellæ, which are particles or droplets of more than normal molecular size, and are large enough to exhibit the properties of surfaces. When the gelatine sol forms a gel, there is no sudden and definite change, but a gradual alteration in a few properties which are conspicuous rather than fundamental; the micellæ are crowded nearer and nearer to each other, until they are perhaps actually in contact with each other; but as they are spherical, each

will be in contact with its neighbours only at a few points on the surface. There will be spaces between the micellæ, filled with water; just as there are air spaces in a pile of cannon-balls. But the separating films of water will be very thin, and most of the water within the membrane will be influenced by surface forces; it will be

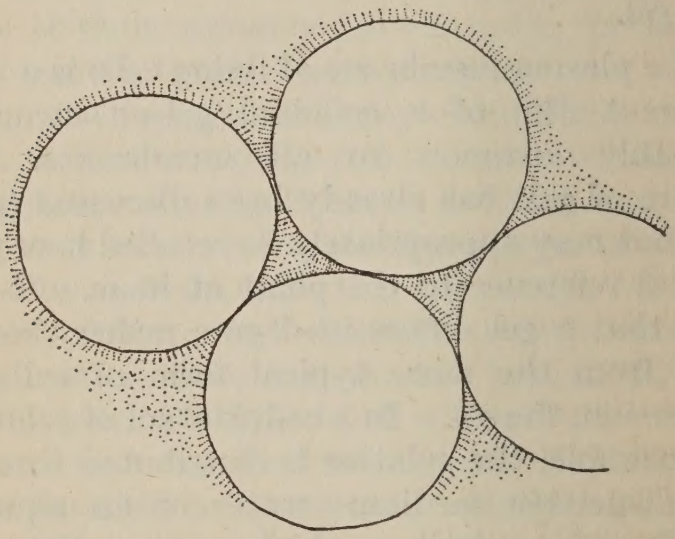


FIG. 3. Structure of a colloidal gel, diagrammatic and magnified about 200,000 times.
Note layers of water molecules adsorbed on the surface of the micellæ.

adsorbed on the micellæ. The properties of a film of water adsorbed on a surface are different from those of water in bulk—we hardly know how different; but at least there will be little movement of the molecules, and a great resistance to the diffusion of dissolved substances.

A thin film of a gel of this nature—a film of colloidal micellæ with adsorbed water, held together coherently most likely by surface forces—that is our picture of the plasma membrane. It is doubtful if there is any justification for drawing conclusions as to the chemical nature of the colloidal micellæ, which indeed need not all be the same, as far as one can see: but it is plausible enough to consider lipoids to be the chief constituents. It is not hard to see that such a membrane permits the passage of water, one molecule taking the place of another in the adsorbed layer, and of sufficiently small molecules, such as those of the gases oxygen and carbon dioxide. The organic compounds which are surface-active will also penetrate well, since they will displace the adsorbed water from the surfaces; it is not surprising to find decreased permeability and consequent narcosis resulting from the treatment of cells with these substances, since they block up the membrane and alter its structure. On the other hand large dissolved molecules which are not surface-active may find it hard to make their way through the pores of the gel, especially if like many inorganic salts they *increase* surface-tension and in consequence avoid the neighbourhood of surfaces; electrical forces may also tend to prevent the passage of ions. There is, indeed, much evidence that indicates that it is the ions

rather than the undissociated molecules which are unable to pass the membrane ; for it is found that acids and bases which dissociate only feebly, as acetic acid and ammonia do, penetrate much more rapidly than the " strong," well-ionized acids like hydrochloric acid, and the corresponding strong bases like sodium hydroxide. As many ions tend to surround themselves with water molecules, they may actually be larger than the molecules from which they arise.

*Ions may considerably alter the structure and properties of the plasma membrane.*

It has so far been taken for granted in this discussion that Overton was correct in considering the plasma membranes as quite impermeable to inorganic salts. But this is no more than a half-truth. If a plant cell is treated with a strong solution of a single salt, such as sodium chloride, it becomes " plasmolysed," the volume of the protoplasm is reduced by loss of water ; after an hour or so, however, the plasmolysis disappears, the protoplasm returns to its normal volume, and it is found that the membrane is no longer impermeable to the salt ; clearly, the ions have in some way altered the membrane so that its permeability is increased ; and if the process goes too far the cell dies as a result. Innumerable instances of the harmful effect of

solutions of single salts have been collected, and it is found that this action is not confined to the plasma membrane alone, but may spread to the internal cytoplasm. Different ions have different effects: the sodium ion causes a liquefaction, a disintegration of the densely-packed micellæ of the membrane into a fluid colloidal sol; calcium ions cause a solidification, a joining together of the micellæ into solid lumps and strands which fall apart, destroying in a different way the coherence of the film. It is not surprising to find that sodium and calcium ions may "neutralize" each other, so that a mixture of sodium and calcium salts is much less harmful than a pure solution of either salt by itself. This principle of the "antagonism" of salts has already been alluded to (page 72), but requires fuller consideration at this point.

In this question attention is focussed on the positively-charged metallic ions, sodium, potassium, calcium and the like; the negatively-charged ions such as chloride and carbonate are not without influence, but much less significant. The reason is that the majority of the colloids of living matter are, under ordinary conditions, negatively charged with electricity, and therefore more affected by the positive ions. It was explained in Chapter II that very concentrated solutions of salts cause colloidal micellæ to lose their stability and independence, so that they

coagulate; this effect is not a simple but a double one. In the first place the positively-charged ions mask or destroy the negative charges on the colloidal particles, and this they do with far greater power if, like calcium ions, they carry twice the usual unit charge of positive electricity; in the second place the ions are surrounded by attracted molecules of water, and in great concentrations may weaken the protective coat of water molecules round the colloidal micellæ, so that, by this double action, the colloidal coagulates or is "salted out." In small concentrations, however, ions may actually increase the stability and independence of the colloidal particles, though *how* they do this is not very certain. An extreme case is furnished by an important group of proteins, the *globulins*, which will not form colloidal sols in water unless dissolved, ionized salts are present; if there is too much salt, the globulin coagulates like any other colloid—more easily, indeed, than some other proteins which dissolve colloidally in pure water! The important point emerges that in solutions whose strength is at all comparable to that of the internal fluid of living cells, the sodium and potassium ions have not reached that pitch of concentration at which the colloids of living matter are precipitated; their action is all the other way, tending to increase the independence and degree of dispersion of the

micellæ, and in consequence to convert dense colloidal gels into fluid sols. Very different is the case of the calcium ions, to which the double charge of electricity gives so strong a coagulating power that even in very dilute solution they decrease the stability and degree of dispersion of natural colloids, and convert sols into gels. The rule is that the liquefying action of the positively-charged ions which carry but a single-unit charge of electricity, sodium and potassium being the most important examples, may be counteracted or "balanced" by the more powerful solidifying and coagulating action of the positively-charged ions which, like calcium, carry more than one unit charge. In sea water, the singly-charged ions of sodium and potassium are balanced by small quantities of the doubly-charged ions of calcium and magnesium; the consequence is that sea-water has very little effect on the colloids of the living cells. In a properly balanced salt solution, such as sea-water, the plasma membranes of cells are not injured and do not become permeable to the ions as they do in solutions of any one salt.

From a large number of possible illustrations of this effect a few may be selected for discussion. Ostwald found that a small amphipod Crustacean, called Gammarus, which usually lives in fresh or brackish water, survived immersion in sea-

water very well; but its life was shorter in salt solutions of any other composition, and shortest of all in solutions of any one pure salt. Osterhout found that grass rootlets would not grow in solutions of sodium chloride or calcium chloride, but grew very well in a mixture of these solutions. Loeb, a leading worker in this field, found that the growth-preventing action of pure sodium chloride solutions on the eggs of a fish could be neutralized by the addition of almost any salt whose positive ion carried more than one unit electric charge, for instance salts of cobalt, lead, zinc, or nickel. But it would be wrong to conclude that in a balanced mixture it is always possible to substitute other ions of the same charge for the calcium ion, or that sodium and potassium are quite interchangeable; the colloidal structure of living matter is far too delicately poised to be insensitive to such alterations in its environment, and many instances are known where one ion or another has a quite specific action. Slight changes in the concentration of one ion or another, either inside or outside the cell, may have unlooked-for effects; the ions undoubtedly regulate the degree of dispersion of the living colloids, and we have yet to study one of the most important applications of this rule.

It is now desirable to compare these experimental results with the theory of the plasma

membrane previously outlined. We have supposed this membrane to be a thin film of a gel, in which colloidal micellæ, possibly of a lipoid nature, are separated only by layers of water so thin that they do not possess the usual properties of water in bulk. We have explained the narcotic action and easy penetration of surface-active substances by supposing that they displace the water adsorbed on the colloid; but since most of these substances are soluble in organic solvents, it is not improbable that they soak into the micellæ themselves, if these really are formed by lipoids. The molecules of gases are perhaps small enough to make their way through between the micellæ in the adsorbed layer of water, but there is little reason why they should not pass through the micellæ themselves. The molecules of many organic substances such as cane-sugar, and of inorganic salts, are unable to dissolve in the lipoid micellæ, and too large to make their way between them; especially when surface or electric forces resist their passage, as is no doubt often the case with inorganic salts. But these very salts may greatly change the character of the membrane, some rendering it too dense, others weakening the hold which each micella has upon its neighbours; with the result that except in a carefully balanced solution, for which the first essential is that the salts with singly-charged positive ion should be " antagon-

ized " by salts whose positive ions carry a double or greater charge, the membrane loses its properties and allows all sorts of substances to diffuse through ; in extreme cases it dies.

The question of the permeability of the cell-membrane to organic dyes has not yet been discussed in these pages, and it may serve now to provide a further illustration of the assumed mechanism of penetration. Overton found that in general the dyes which " vitally stained " living cells, that is, those which made their way in from the exterior, were nearly all soluble in lipoids ; he regarded this as one of the strongest points in support of his view that the plasma membrane was a continuous film of lipoid. But it has since been found that there are dyes soluble in lipoid which do not stain living cells ; this is probably because they form not true but colloidal solutions, and have micellæ so large that they cannot get through such outer investments as the cellulose walls of plant cells ; in fact most of these dyes do stain any animal cells which are not protected by an outer covering of some sort. It may in general be said that the dyes which are soluble in lipoids, and which stain living cells, are " basic " dyes, that is salts of a complex organic base with an inorganic acid. There is little to suggest that these dyes are surface-active substances, and the writer thinks that they pass the plasma membranes by

dissolving in the lipoid material of the micellæ ; their molecules are perhaps too large to pass through the interstices of the gel.

At first sight difficult, but on close inspection very instructive, is the fact that there are some acid dyes which are insoluble in lipoids and yet penetrate into cells. But they penetrate into cells only of a certain kind : if a frog is stained with a dye (e.g. cyanol) of this sort, the tissues as a whole remain colourless ; the dye appears only in certain cells. The cells stained are of two kinds : first the " phagocytes " of the blood and tissues, cells which like the amœba have the power of engulfing solid particles, and must be supposed to have rather peculiar surface properties ; second the cells of the kidneys, through which large quantities of inorganic salts and other feebly-penetrating substances most certainly pass all day long, and which again must be supposed to have surfaces adapted to their particular function. It is significant that narcotics prevent these acid dyes from entering the cells, while they do not affect the penetration of the lipoid-soluble basic dyes ; Höber is surely right in suggesting that the specialized cells into which the acid dyes enter have some peculiar means of permitting or even encouraging the passage through their membranes of substances to which such membranes are usually impermeable.

The cells in which activity of this sort is best known are those which line the small intestine in mammals, and whose function it is to draw the digested food-stuffs from the alimentary canal and pass them on to the blood. It is likely, though some of the facts are still in dispute, that this is not simply a process of diffusion, or controlled merely by osmotic pressure. If two exactly similar salt solutions are separated by a *living* sheet of cells from the intestinal wall, a distinct movement of solution through the cells can be shown to take place ; this can only be accounted for by the supposition that the cells, actively and with output of energy, draw in the solution on one side and force it out on the other. This will even go on in spite of a very considerable osmotic pressure acting in the opposite direction ; it cannot be a property of the cell-membranes, but a property of the cells themselves, that in some unknown way they act as pumps. On the other hand, it is likely that the membranes of these cells are peculiar, in that they are more than usually permeable to inorganic salts and organic compounds such as sugars and amino-acids. Naturally the usual relations hold : surface-active compounds pass more rapidly than others, and if osmotic pressure helps instead of hindering, it is so much the better. The case of the kidney cells, through which pass waste products and inorganic salts,

against an osmotic pressure, is still clearer. Of the many facts established in the study of the phagocytes, very few help us to a clear idea of the nature of their surfaces or the reasons for their peculiar properties.

It was at one time thought that the interior of the cell was divided up into little compartments, in each of which a different chemical reaction might be going on. This view was well received in the days when it was not realized that the net-like appearance of fixed and stained protoplasm fails to correspond to the living reality. It is true that even living protoplasm is crowded with little vacuoles, each of which is probably lined with a plasma membrane; perhaps the membrane which marks the boundary of the nucleus is very similar; and it may be that some of the solid inclusions in the cytoplasm have a similar investing layer; but microdissection has rather exploded the idea that the living protoplasm itself is actually partitioned into compartments. Nowadays it is easier to see how different chemical reactions may proceed simultaneously in one fluid, for if that fluid is the aqueous dispersion medium of living matter it bathes many different surfaces, at which different reactions may be catalytically promoted. Warburg's experiments, described on page 87, showed that when the structure of the cell was destroyed by grinding, the con-

sumption of oxygen and output of carbon dioxide fell to a low value, and there is very probably great chemical confusion in the fluid mixture. Interesting in this connection is the suggestion of Warburg and Osterhout, that cells have to spend a certain amount of chemical energy merely in keeping their structure and their surface membranes intact and in good repair ; it is hard, indeed, to see what some cells do with the energy they use up. It is not impossible that active energy-consuming import and export of dissolved substances may take place even in cells which are not specialized for the purpose.

*All living cells are irritable ; they respond in a definite way to stimulation.*

All living cells are *irritable.* The word in this connection has nothing to do with either bad temper or discomfort ; it means that the cells respond to certain changes in their surroundings by an alteration in their activity. Let us clearly distinguish two different things : a plant cell responds to an increase in the osmotic pressure of the surrounding medium by shrinking (plasmolysis), but that is not a change in activity ; it is a purely passive change. An amœba reacts to an electric shock, or to the touch of the micro-dissector's needle, by withdrawing any

projecting parts of its protoplasm and making itself nearly spherical; this is a change in activity, displaying the irritability of the cell. A change in the environment which produces an active reaction in a cell is called a *stimulus*. It is hard to avoid circular definitions in this connection, without saying less or more than the truth.

There are many different kinds of stimuli. Electric shocks, or sudden changes in the flow of an electric current, stimulate all cells; otherwise, there is much specialization. The cells of the retina of the eye are stimulated by light; of the cells in our skin that communicate with the nervous system directly, some are sensitive to increases, others to decreases of temperature; others to mechanical pressure. The green cells of the plant are sensitive to light: but the cells of the leaf of *Dionœa* are sensitive to the weight of an alighting fly, and the leaf snaps upon its victim like a closing book. The muscle cells of the uterus of a guinea-pig which has previously been treated with the serum of a horse react with violent contractions to the presence of the least trace of a similar serum, but not at all to the serum of a cow. Most marvellous instance of all, the egg-cell of the frog reacts, not only to the entry of the spermatozoön but to all sorts of different stimuli: without being fertilized at all it may commence the long sequence of

orderly divisions and subdivisions which at long last produces the tadpole.

It is rather characteristic of the reactions of cells that whatever the stimulus, the response is the same. This is certainly true of the specialized cells: a muscle reacts by contracting to a nerve impulse, to an electric shock, to sudden heat, to a suitable salt solution, to mechanical injury; many stimuli, one response. A gland cell secretes, a sensory cell sends out a nerve impulse, an egg-cell divides. The response may be not merely always the same in kind, but always the same in quantity; if a stimulus is sufficient for a muscle cell to respond at all, it responds with all its might. This is the "all or none" law, and it is probably of wide application. No doubt when a *tissue* is examined it may be hard to say whether all the cells respond equally but feebly, or a few cells respond vigorously in agreement with the law; the law is true only of single cells or cells whose protoplasm is continuous. Another characteristic feature of irritability is that immediately after a response has begun there is a brief "refractory" period during which the cell ignores any further stimulus. On the other hand if a stimulus too weak to evoke a response is closely followed by another, the combined effect may do what neither could do alone; this is called "summation of stimuli." Let us also note that the characteristic effect of

narcotics is to abolish irritability, and that a similar unresponsive state may be arrived at more naturally; this is called "inhibition."

*When a cell responds to stimulation, the plasma membranes become more permeable.*

It is very clear that in a large number of cases the cell's response to stimulation involves an increased permeability of the membranes. When the sensitive plant *Mimosa* responds to the stimulus of a gentle touch, the visible movement of the leaves is due to a decrease in the turgid rigidity of some of the cells; this decrease in internal pressure is traceable to an escape of fluid from the interior of the cells, and the fluid which escapes is water with salts dissolved in it; the whole response of the sensitive plant (we are not discussing the conduction of the stimulus from part to part) is dependent on an increased permeability of some of the cells. Few cases are as clear as this; but there is enough evidence to make it a reasonable as well as an attractive idea that increased permeability is the indispensable precursor of the response of cells to stimulation. It is even allowable to define a stimulus as a change in the environment producing an increase in the permeability of cell membranes, and response as the direct and indirect results of this increase. Inhibition, on

the other hand, implies a decrease in permeability, such as narcotics also produce.

An isolated strip of muscular tissue contracts when it is pinched : it is likely enough that the delicate plasma membranes have sustained some mechanical injury. A muscle will also contract energetically in a solution of any single salt, such as sodium chloride, even if no osmotic forces are brought into play ; and we have just discussed at some length the increase in permeability brought about by such solutions. The entry of the spermatozoön into the unfertilized egg-cell must obviously affect the surface, and there is excellent evidence that an increased permeability over the whole surface of the cell results ; all the various artificial means (to be discussed later) of evoking the complex response of the egg-cell seem to depend on surface influences. When we turn finally to the most important and most general case of stimulation and response, stimulation by electric currents, the significance of surface properties meets us at every step.

When an electric current passes through a solution, the electricity is carried by the ions ; the positively-charged ions move in the direction of the positive current through the water, the negatively-charged ions in the opposite direction. In this way there comes about a partial separation of the two kinds of ions, the energy required

to pull them apart in spite of the attraction which the opposite electric charges have for each other being supplied by the electric current; if the separation is carried far enough, it may often lead to marked decomposition ("electrolysis") of the dissolved compound. When an electric current is passed through a living tissue, it is similarly carried by the ions, which will proceed to move in the direction indicated by their electric charges. But the tissue is divided up into cells, and the cells are bounded by membranes through which the ions are unable to pass; it follows that in time the positively charged ions will find their progress stopped by the membrane at one side of each cell, and will tend to accumulate there, while the negative ions will be concentrated at the other end of the cell. One result of this is that the ions, being unduly concentrated in this way, will attempt to diffuse back from the boundaries of the cell, against the electric current; in this way a considerable resistance to the passage of the current through the cells is produced, and is called "polarization." The second result of such a concentration of ions can easily be foreseen: it will inevitably alter the degree of dispersion of the colloids, and particularly the permeability of the colloidal plasma membrane. The permeability of the cell will be increased by the passage of the electric current. According to Nernst and his

followers, the " polarization " at the cell boundaries and consequent changes in permeability of the membranes and colloidal dispersion are the only *immediate* results of stimulation by an electric current. It is possible that the hydrogen ions of acids and hydroxyl ions of bases are even more important than the ions of salts, but this does not affect the main point. The more conspicuous results of electric stimulation, such as the powerful contraction of muscle cells or the increased secretion of gland cells, are only *secondary* to the colloidal changes which are produced by the disturbance of the normal balance of the ions in the protoplasm. Here, unfortunately, there is a gap in our knowledge ; it is not known just how the colloidal changes lead to the formation of lactic acid in the case of muscle, for example ; but it is easy to guess that the breakdown of a colloidal partition allows the " lactacidogen " to come in contact with an enzyme that promotes the reaction. It is difficult to find out *how* a colloidal change leads to a chemical reaction, but not difficult to see *why* it should.

It is therefore fairly well established that electrical stimulation depends firstly on an increase in the permeability of the cell ; and that the same may be said of all types of stimulation is an opinion widely held. It follows, in the words of R. S. Lillie, that " the plasma membrane

is not merely a protective layer but plays the part of a sensitive intermediary between the external world and the internal protoplasm." This point is emphasized by a famous experiment: certain cells were treated externally with a solution of the drug *muscarine*; it was found that so long as the drug was diffusing through the plasma membrane, which was permeable to these molecules, the action of the drug on the cells was well marked. But as soon as the diffusion was over, and the internal concentration of the muscarine molecules was equal to the external concentration—as soon, in fact, as the inflow of the drug through the plasma membrane had ceased—the cells recovered completely and showed no trace of the presence of the drug within them. It may further be observed that it has been suggested that some of the chemical reactions usually considered to take place within cells may actually take place at the surface of the plasma membrane; and there is no good reason for denying that this may often be the case.

*There is a delicate balance of electric potentials in living tissues, which is disturbed in response to stimulation.*

It has so far been assumed in these pages, tacitly at least, that because the plasma membranes are usually impermeable to dissolved

inorganic salts, they are necessarily impermeable to the positive and negative ions alike ; but that is by no means the case. For if the membrane is permeable to only one of the ions, it will, practically, be impermeable to the salt as a whole. In the case of a cell whose interior is rich in potassium chloride, and which is immersed in sea water, or any other balanced solution which does not contain much potassium—the lymph of the tissues being an excellent example —let it be supposed that the plasma membrane is permeable to the potassium ion but not to the chlorine ion, nor to any of the other ions present. Then certainly as the potassium ions wander to and fro within the cell, sharing in the restless activity of the water molecules, they will at times pass through the membrane to the exterior. But as soon as they do this there arises a difference of electric potential, since there are more negative than positive ions left inside the cell ; and the attraction between unlike charges of electricity prevents the positive potassium ions from escaping altogether ; Bayliss has suggested the comparison of this to a field in which are ewes and lambs in equal numbers, and which is enclosed by a fence through which the lambs can pass but the ewes cannot, so that, although lambs do wander outside, they never go far away and always return at length to their mothers. Yet there

will ordinarily be more lambs outside, more ewes inside; and in the same way there will ordinarily be more negative ions inside the cell, more positive ions outside the cell, in the case that has been put forward above. That is to say, there will be an uneven distribution of electric charges, or in other words a difference of potential between the interior of the cell and the exterior.

Consider now the effect on the system of increasing the permeability of one part of the cell membrane, so that other ions are able to pass through. Before this, the potassium ions inside attempted to escape but could not overcome the electrostatic attraction of the negative ions they left behind; while the sodium ions which were more concentrated in the external medium than inside the cell attempted to make their way inwards through the membrane, but could not because the membrane was impermeable to them. Now, however, the membrane has become permeable to the sodium ions at one point; and consequently, the way standing open before them, the sodium ions pass into the cell. As they pass in, the sodium ions carry with them charges of positive electricity: and these positive charges "satisfy" the negative charges left in the interior of the cell by the wanderings of the positive potassium ions. Consequently, as fast as the sodium ions enter

the cell through the weakened part of the membrane, the potassium ions are set free to escape from the rest of the membrane into the external medium, where they in turn "satisfy" the negative ions left behind by the sodium ions entering the cell. The two processes, entry of

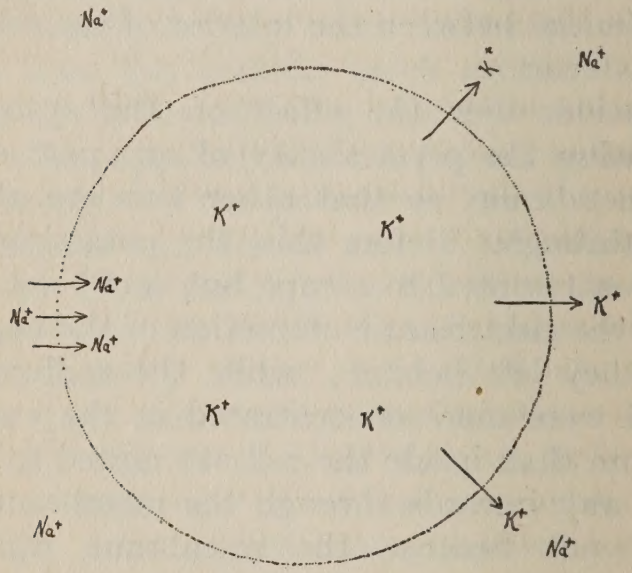



FIG. 4. Diagram illustrating electric currents in living cells. The sodium (Na) ions outside enter the cell where the membrane is weak, allowing the potassium (K) ions to escape elsewhere.

sodium ions and compensatory escape of potassium ions, are interdependent; without the one, the other could not take place. But the net result is that some positive ions are entering the cell at one point, where the permeability has been increased, and other positive ions are leaving the cell at another part of the surface;

there is therefore a flow of positive electricity, an *electric current*, through the cell; entering where the membrane is weak, leaving at other parts of the surface.

To detect such a current, it is necessary to complete the electric circuit artificially; a suitable electrode is applied to the weakened part of the membrane, another to the surface of the cell elsewhere, and the two are connected by wiring in the ordinary way, with a current-measuring instrument (galvanometer) in the circuit; it should then be found that a current passes through the instrument. Within the cell, the positive current travels from the injured or weakened part of the membrane to the other, untouched parts; the injured part, from which the positive current starts, is then said to be "negative" with respect to the rest of the cell. Now it must be admitted that so far few measurements have been made on single cells; but what is true of single cells should be true of a number of cells together; and it is found that when a muscle fibre is cut across, there appears the so-called "injury current," which has the direction that agrees with this theory; the injured part is negative with respect to the rest of the fibre. The same current appears in the case of an injured apple. Quite apart from violent injury, however, it has been shown that stimulation of a cell causes an increase of

permeability; and a stimulated cell ought to show an electric current in the same way and in the same direction. This, in fact, is a well-known truth; *every* response to stimulation is accompanied by an *electric* response, and the stimulated part of the cell or tissue becomes negative; this is called the "current of action."

Let us re-examine our theory of these currents. It depends on two main premises; that there are more sodium ions outside, and more potassium ions inside; and that the potassium ions are unlike all the others present, positive or negative, in being able to penetrate the membrane. For if there were no difference between the two fluids, the internal and the external, the ions would have no impulse to diffuse; the source of the energy of the current is the energy with which the ions attempt to diffuse through the whole available volume of solution, and is, as was explained in connection with the theory of osmotic pressure (page 36), comparable to the pressure of a compressed gas. On the other hand, if the potassium ions were not able to pass through the plasma membrane, no current could arise; for when the membrane became permeable on injury or stimulation, the sodium ions would doubtless enter the cell, and the potasium ions leave the cell, but they would both be passing through the weakened part of the membrane, the positive charges going

through in one direction would exactly equal the positive charges going through in the other, and there could be no *current* of positive charges, but only a to-and-fro movement. Our theory in brief is, then, that the potassium ions are always trying to leave the cell, but can only do so when the plasma membrane is so weakened by stimulation or injury that it becomes permeable to sodium ions in the external medium; if the weakening is confined to one part of the cell, and the potassium ions can escape from other parts of the cell, then there will be a flow of positive electricity, and the weakened part of the membrane will become relatively negative.

A cautionary parenthesis may be inserted here. The foregoing theory is built largely on Bernstein's studies of muscle cells; it explains fairly well the injury currents and currents of action, it gives a straightforward picture of the formation of currents in living cells and the alterations in electric potential which accompany stimulation, and it does not seem to conflict with any reliable facts. But it makes many assumptions, and there is no good proof of a direct kind that cell-membranes are permeable to potassium and not to sodium (though as the potassium ion is a *smaller* body in solution than the sodium ion, it is not an unreasonable idea), nor that they are permeable to potassium ions and not to chloride or other negative ions; indeed, the

red-blood corpuscles seem to be permeable to negative rather than positive ions. But the principle of the theory is not greatly altered by supposing, with R. S. Lillie, that it is negative rather than positive ions which travel in these currents, and the reader will have no difficulty in transposing the theory to suit this case if he prefers it. Most of the experiments of an electrical nature, made especially on muscle cells and on the apple, and on suitable artificial models, give the important rôle to the positive ions, sodium and potassium, and that view has been selected accordingly. It may further be observed that this theory of electric currents depends on the theory of cell-membranes adopted before, and that in turn on the theory of the structure of colloidal gels which has been upheld throughout this book; it may seem a top-heavy house of cards, but the author hopes that at least it is continuous and coherent, and nowhere in conflict with established fact, nor with any overwhelming majority of opinion.

In some cases the electrical current which follows stimulation must be regarded as an apparently useless accompaniment to the physiological response. In a few cases cells have been specialized to produce enormously powerful electrical currents when stimulated, in the "electric fishes," to which the voluntarily controlled current is a valuable weapon of offence

and defence. But by far the greatest significance of the electric currents of living cells is that they pass on stimulation from cell to cell. In stimulation there is always a "local" effect and a "propagated" or spreading effect. If a nerve is stimulated at some point, the effect is propagated down the fibre to the muscle, if it is a motor nerve, or to the brain if it is a sensory nerve. There is no reason to invoke unknown forces to explain the nerve impulse; it is a variation in electric potential that is passed on from cell to cell, from nerve fibre to muscle or gland cell or brain cell; the current from one stimulates the next. We are reminded of the walruses that sleep in rows on the edge of the ice-floe; when one is awakened by a push from its neighbour, it stirs and looks around to see that all is safe, and before sinking back to sleep pushes and wakes its neighbour on the other side. Each cell gives its characteristic response to stimulation, and passes on the stimulus to its neighbour; the tissues are electrically co-ordinated. Quite recently it has become clear that there is a chemical side to the transmission of the nervous impulse; oxygen is used up, carbon dioxide liberated, heat is produced. Nevertheless, it still seems desirable to regard the propagation of differences of electric potential as primary, the chemical reactions as secondary processes, possibly of repair or restoration.

It may be desirable to conclude this chapter by surveying a few of the characteristic features of the conduction of the nervous impulse. We have seen that the typical nerve cell is brought, by means of branching processes, into close relation with its neighbours, and that it possesses one main process, the *axon*, which may be some feet in length. Along an axon the nervous impulse travels at a definite rate, which is independent of the strength of the stimulus which started it; in man, the rate of conduction is over 400 feet per second, but it is a curious and difficult fact that in the lower animals it may be very much less. The strength of the stimulus has no effect even on the strength of the impulse, which is always the same; the axon obeys the " all or none " law. This is well shown by the experiment of depressing some portion of a nerve fibre, by means of an anæsthetic: if the portion of fibre so treated is long enough, an impulse is unable to make its way through the depressed region at all; but if the length of the portion is just a little shorter, so that the impulse is just able to get through, then as soon as the impulse has entered the unaffected fibre beyond the depressed region it regains all its original strength and shows no sign of having been nearly extinguished. We may compare with this the behaviour of a train of gunpowder; the rate and the strength of the

wave of explosion that travels along it is independent of the " stimulus " that was used to set it off ; if at one part the powder is somewhat damp, the wave may be choked out altogether, but if it does get through to the dry powder beyond it flares up again to its original strength. Again, a single axon, like a train of gunpowder, will conduct an impulse equally well in either direction. The analogy, however, must not be pushed too far : it is clear that in the train of gunpowder we have a *transmission of heat* by means of chemical reactions, oxidations ; in a nerve fibre we have a *transmission of electric potential*, whether by means of oxidations or not we cannot say.

Considerable interest attaches to the question of the passage of the impulse from one nerve cell to another ; for instance, a " sensory " message from the skin to the brain travels along a chain of three or more nerve cells. The exact structural relations of the branching processes of one cell to those of another, at the so-called " synapse," are still somewhat obscure, but it is generally believed that there is not an actual continuity of protoplasm from cell to cell. In any case, the synapse has distinctive physiological properties : it delays the passage of the impulse ; it is subject to fatigue ; it is affected by certain drugs ; it does not accurately obey the " all or none "

law, but may add two stimuli together; and—most important—it only allows impulses to pass through in one direction. Hence, although each axon can conduct in either direction, it is impossible for a message to travel from the brain to the skin by the same path as a message in the reverse direction, because each synapse acts as a valve, permitting impulses to pass through only in one predetermined direction (in general, impulses travel into a nerve cell by the short processes, and from the centre of the cell down the axon). This is one of the salient facts of the physiology of the nervous system.

# CHAPTER V

## THE LIFE-HISTORY OF THE GERM-CELLS

*Each new life typically begins with the fusion of two germ-cells, one paternal and one maternal.*

ALL cells arise from cells previously existing, which divide in an elaborate and orderly way, described in the first chapter of this book. In the body of a grown man there may be perhaps twenty million million red-blood corpuscles; these were formed by the division into two of *half that number* of antecedent cells, and these again by the division into two of earlier antecedent cells. As the number of cells, enormous though it is, is halved at each division as we look backwards, we soon come to very small numbers indeed, and eventually to one single cell from which, in a multitude of cell-generations, has arisen the entire cellular fabric of the body. Let us recall the story of the Eastern ruler who rashly promised to put a grain of corn on one square of a chess-board, two on the next, four on the next, and so on; only to discover that the resources of his kingdom were too limited

for him to supply the required nine million million million grains of corn for the sixty-fourth square !

Towards the end of the present chapter, some space will be devoted to a consideration of the first few divisions in the long sequence by which the complex body of the higher animals and higher plants is built up from a single cell ; but before this it will be necessary to consider the origin and descent of this initial single cell. In most cases this single cell, the fertilized egg-cell, is formed by the orderly union of two cells, one paternal and one maternal—in animals the spermatozoön and the ovum. This process, the fertilization of the maternal ovum by the paternal spermatozoön, is the essential feature of sexual reproduction. With asexual reproduction, which is seen when a whole sea-anemone divides into two similar halves, or when a strawberry plant sends out a runner on which new plants grow, this book is not concerned ; asexual reproduction is an affair not of one cell but of many, except in the case of the division by fission of the one-celled animals and plants, which will be noted hereafter. We are at present concerned with the properties and the origin of the two cells, spermatozoön and ovum, the *gametes*, as they are often called.

*One of the cell-divisions preceding the formation of the gamete of either sex is peculiar, since it involves a reduction in the number of chromosomes.*

The number of spermatozoa produced by a male animal may be vast beyond computation; but they are all descendants of a single cell, which is in turn one of the immediate descendants, at but few removes, of the original fertilized egg-cell from which the whole body was gradually differentiated. This ancestor of the spermatozoa may be called the primordial germ cell; and from it there arise, one generation of cells after another, the *spermatogonia*, the last of which divide to form the *first spermatocytes*. These first spermatocytes may be considered to be the cell-grandparents of the spermatozoa; there is but one intervening generation of cells—the second spermatocytes.

All the divisions up to and including that which produces the first spermatocytes are of the typical kind described in the first chapter; but it is now necessary to re-examine this typical process and to give special attention to the chromosomes. It was explained that within the resting nucleus, which is apparently almost without structure, there appears as a preliminary to cell-division a coiled thread, the *spireme*. This thread carries upon it the chromatin, the

stainable material, irregularly distributed along the length of the thread in granules. The thread soon splits up into segments, each of which becomes shorter and thicker, till it is a single dense rod-like *chromosome*; in typical cell-division, each of these chromosomes divides along its length into two—there may be a hint of such longitudinal division at a very early stage in the formation of the spireme—and half of each chromosome then goes to each daughter-cell. When a nucleus divides into two, the chromatin is divided between the two resulting nuclei with scrupulous accuracy.

The significance of this becomes clear when it is explained that for each species of animal or plant the chromosomes show a very remarkable constancy. Usually in each cell throughout the whole body, certainly in the primordial germ-cells and their descendants, the number of chromosomes is constant and characteristic of the species. Counting may be difficult, for the chromosomes are very small, and sometimes observers disagree: but in man the number of chromosomes is said to be forty-eight in each cell, and the same number is reported for the snail and the banana; in one variety of the thread-worm of the horse there are only two chromosomes. Not only is there constancy of number, but there may be great constancy of *shape*; some of the chromosomes may be dis-

tinguishable by some peculiarity of shape and size, and in that case these recognizable chromosomes will be found in every cell, not merely in the individual but throughout the species (a few exceptions to this rule will be dealt with later). In this connection it should be observed that such identifiable chromosomes always occur in *pairs* ; if one has some peculiarity, another will be found in the nucleus to match it. There is every reason to believe that each chromosome has a " partner," and that of these pairs one partner traces its descent right back to the spermatozoön, the other to the ovum ; one to each of the two gametes from whose union into one cell the whole body arose. In short, one is paternal and one maternal. Furthermore, there is at least one clear case in which the constancy of the chromosomes does not stop short at number or shape or size, but is expressed in their minute structure : Wenrich was able to compare corresponding chromosomes from the cells of a species of insect, at an early stage in their formation, when they were not yet compact but only thin segments of the spireme with little lumps of chromatin scattered along the length of the thread ; he found that in each of thirteen individual insects not only could this same early chromosome be recognized, but in each case the lumps of chromatin (" chromomeres ") were distributed along the thread in exactly the same

way ; a lump at one point on the thread in one individual always corresponded to similar lumps in the other individuals. Let us sum up the position : when each first spermatocyte is formed, it contains chromosomes which in their *number*, *size*, *shape*, and probably *structure* are constant and characteristic for the species. One is unable to refrain from concluding that although the chromosomes disappear in the resting nucleus, between divisions, they are not broken up and disintegrated ; the constancy of their reappearance is too great for that to be possible, one would think ; and it is more likely that by some change in colloidal dispersion they become invisible, than that they actually lose their individuality and fine-grained structure.

Now it is obvious that if the gametes themselves carried the full number of chromosomes typical of the species and found in the first spermatocytes and corresponding female cells, then when ovum and spermatozoön united to form the fertilized egg-cell this would contain twice the normal number of chromosomes ; and unless there were a fusion of chromosomes in the nucleus after fertilization, which there is not, all the cells of the next generation would have a double supply of chromosomes ; and the generation after that would have four times the original number, and so on, *ad infinitum*. This is manifestly impossible ; and we are not sur-

prised to find that between the formation of the spermatocytes and the formation of the spermatozoa there takes place a *halving* of the number of the chromosomes, and the same thing occurs in the formation of the ovum. The result is that each of the gametes has only half the typical number of chromosomes, and the fertilized egg-cell starts its life with the full or proper number of chromosomes, half of these having come from the male side and half from the female side. It is usual and convenient to call the cells in which the number of chromosomes is halved *haploid* (single), while the body-cells, the first spermatocytes, and the fertilized egg-cell —all those, in fact, with the complete number of chromosomes—are called *diploid* or double.

This reduction in the number of the chromosomes takes place when the first spermatocytes divide to form the second spermatocytes, which they do, naturally, in a rather peculiar way called *heterotypic*, *meiotic*, or *reductional* division in contrast to the usual *homeotypic*, *mitotic*, or *equational*. The process is a complicated one, but its essential features are of such importance that they repay study. The first spermatocyte is a short-lived cell, and its nucleus never passes into a completely structureless state. Soon the early chromosomes, in the form of thin threads with lumps of chromatin scattered along them, become distinguishable; there is still the

full or diploid number. These thin chromosomes then unite together in pairs, lying in effect side by side and closely apposed. It is reasonably certain that the chromosomes do not select their partners at random, that each comes into contact with its true "opposite number." The process of the union of the chromosomes is called "synapsis," and the partners are "synaptic mates." Of each pair of partners one is derived from the male parent, and one is the descendant of the corresponding chromosome from the female parent. The synaptic union of the chromosomes in pairs is only temporary, but of much importance, for there may take place at this time a certain interchange of material between the synaptic mates, as will be seen later.

After synapsis, the chromosomes, now only haploid in number, become denser and thicker; often they bunch together in a knot in one part of the nucleus, making observation very difficult. There follows a retrograde phase whose meaning is obscure; the chromosomes become thinner again and lose their sharp outlines for a time, but again return to the characteristic dense form. At this stage the chromosomes are known as "tetrads," for they have a quadruple structure more or less clearly visible. They may be compared to a candle which is first split in half down its whole length, with a knife, then

split again into quarters, again down the whole length, but this time with a cut at right angles to the first. The meaning of these two divisions is clear: one corresponds to the line at which the pairs of chromosomes originally united, the other corresponds to the line at which the halves will split in a future division. At this stage, then, the first spermatocyte nucleus contains a haploid number of " tetrad " chromosomes. Nuclear division now sets in, the tetrads assemble at the equator of the spindle, and the chromosomes split in half along the first of the two lines of division mentioned, so that each half corresponds to one of the two synaptic mates. The halves separate, and the second spermatocytes are formed: each contains the haploid number of chromosomes. Of the synaptic mates that formed the tetrad, one has gone to each second spermatocyte; but it must not be thought that all the " paternal " partners have gone to one daughter-cell, all the " maternal " partners to the other; that is a question which each tetrad decides for itself and by chance. From one pair of synaptic mates the paternal partner may go to the left, the maternal to the right; the next pair of synaptic mates may distribute themselves in the opposite way. But each spermatocyte has got one member of each pair of synaptic mates.

The chromosomes in the second spermatocytes

are split along their length, all this time, by the second line of division of the tetrad; but this has no immediate effect. However, these second spermatocytes are again very short-lived cells, and very soon divide to form *spermatids*. This division is a perfectly normal one, except that there is only half the usual number of chromosomes to distribute; each one splits along the line of division that appeared so long before, so that half of each one goes to each spermatid. The first spermatocyte, with its diploid number of chromosomes which united in synapsis to form tetrads, has now given rise to four spermatids, each of which has the haploid number of chromosomes; each tetrad is divided among the spermatids. Let us consider the whole history again, for clearness' sake, and follow the adventures of one of the original chromosomes of the first spermatocyte, let us say one descended from the female side: in synapsis it found its partner descended from the male side, and came into intimate contact with its mate; in this contact it underwent several changes of shape and developed an incipient division; it was then torn apart from its synaptic mate, which went in the opposite direction; it became for a short time one of the haploid chromosomes of the second spermatocyte, but soon divided again in the direction already forecasted; half of it went to one spermatid, half to the other.

The spermatids are rounded cells of moderate size; but they become profoundly altered in shape and size and structure, to form the spermatozoa without any further division. The spermatozoa are highly specialized cells, varying enormously from one species to another, but mostly built upon one typical plan, as a very long, thin cell with a *head*, a *middle piece*, and a long and slender *tail* or *flagellum*. The head contains the nucleus, a small dense mass of chromatin in which no structure can be discerned; and often a protecting cap in front: the shape of the head is that of an elongated egg. Next comes the middle piece, as long as the head but thinner and nearly cylindrical; it contains the centrioles and a small amount of cytoplasm with, no doubt, representatives of the mitochondria and Golgi bodies. The tail is still thinner, and perhaps ten times as long as all the rest; it lashes to and fro, and gives to the sperm as a whole its characteristic power of active movement. The most curious exceptions to this general type of sperm are found in the shrimps and crabs and other Crustacea, where the sperms are rounded or star-like and very sluggish in their movements; in thread-worms again the spermatozoa are blunt cones, slightly amœboid; but with these peculiar forms we need have no more to do.

*The ovum develops in much the same way as the sperm, as far as its nucleus is concerned.*

When we turn from the origin of the male gamete to that of the female gamete, we find that as far as the nuclear divisions are concerned there is the greatest possible correspondence between the two sides. The *oögonia* divide in the usual equational way to form *oöcytes* or immature states of the egg-cells; the nucleus of the diploid oöcyte then divides reductionally to form two haploid nuclei, in exactly the same way as the first spermatocyte nucleus does; these two haploid nuclei then divide again, equationally, to form *four* haploid nuclei perfectly comparable to the four nuclei of the spermatids descended from one first spermatocyte; synapsis of chromosomes takes place on the oögenetic line just as much as on the spermatogenetic line. But in the female line we meet with quite a new feature, not in the division of the nuclei, but in the subsequent division of the cytoplasm. In the divisions of the spermatocytes, the cells produced are of approximately equal size; in the divisions of the oöcyte, one of the cells is extremely small, the other very large. This may be regarded as an adaptation towards the production of the typical ovum, which is an unusually large cell, rounded, sluggish in movement and in chemical activity, and

heavily charged with a store of food-materials of all sorts—proteins, fats, more rarely glycogen —conveniently termed the yolk.

Even before the nucleus of the oöcyte begins to divide, there may be a growth of the cell at the expense of neighbouring cells. The oöcyte is frequently invested with a layer of " follicular " cells, which are perhaps best regarded as potential oöcytes diverted from their normal development to supply the needs of one cell which has been selected as the representative of the group. The oöcyte grows, and the follicular cells shrink ; material passes from the latter to the former. At length the oöcyte escapes from its surrounding follicular cells and enters upon the series of divisions called the " maturation " of the ovum. The first of these divisions is a reduction division, preceded by a synapsis of the chromosomes and the formation of tetrads, and leading to the formation of two haploid nuclei. One of these remains in possession of almost the whole bulk of the cytoplasm of the oöcyte ; the other travels away to the surface, and there forms a very minute cell, the *first polar body*. The haploid nucleus in the oöcyte divides again, and again one of the resulting nuclei remains in possession of almost all the cytoplasm, again the other is cast out to form a very minute cell, the *second polar body* ; while this is going on, the first polar body usually divides into two, so

that four equal haploid nuclei arise from the diploid oöcyte nucleus; but one is surrounded by a large volume of yolk-laden cytoplasm, the other three have hardly any cytoplasm at all, and presently degenerate and perish.

When the maturation is complete and the polar nuclei have separated, the haploid nucleus of the ovum is ready to unite with the haploid nucleus of the spermatozoön. It is very commonly found that the spermatozoön has entered the cytoplasm of the ovum, in short that fertilization has been accomplished, before maturation is over; many oöcytes, indeed, do not mature unless and until they are first stimulated by the entrance of a sperm.

*Fertilization involves physiological as well as structural changes.*

The female sea-urchin pours forth enormous numbers of ova into the water, the male sea-urchin even greater numbers of sperms; a chance meeting between an unfertilized ovum and a sperm leads to fertilization, and the life of a new individual begins. Some authors believe that the ovum secretes some chemical substance which attracts the sperms. In other animals there are often very elaborate precautions for ensuring that the ova and the sperms shall come in contact; fertilization takes place, for instance, within the body of the female. In

all cases, however, fertilization depends mainly on the motility of the sperm, which at last finds its way to the ovum.

The ovum is encircled by a delicate " vitelline " membrane, probably rather denser than the ordinary plasma membrane; there may be special weak spots through which alone the sperms can enter. The entry of the sperm into the cytoplasm is not due solely to its own activity; the ovum actively helps to draw in the sperm. Chambers has recently described a very remarkable case of this sort in the eggs of a starfish, which are surrounded by a layer of jelly; here the sperm is drawn through the jelly and through the vitelline membrane by an outgrowth from the cytoplasm of the ovum. As soon as the sperm has entered into an ovum, a whole series of changes take place; in some species one type of change is more noticeable than another, but the general features are very constant.

The first reaction of the ovum to the entry of the spermatozoön is that the vitelline membrane becomes separated from the underlying protoplasm, probably first at the point where the sperm entered, but very soon around the whole surface; the ovum now floats in a " perivitelline " space filled with a clear viscid fluid and bounded by the outer " fertilization membrane "; membrane separation is perhaps the

most certain sign that fertilization has been accomplished, and its effect is to prevent any other sperms from entering the ovum; the surface of the ovum is now said to be blocked. At the same time there is undoubtedly an increase in the permeability of the surface of the ovum to dissolved substances; this can be tested experimentally, and sometimes shows itself naturally by the escape of dissolved colouring matter from the cytoplasm of the ovum: correlated with this is an increased susceptibility to external changes, such as the introduction of drugs or narcotics into the medium or alterations in the concentration of the ions. At the same time, too, there takes place a very conspicuous increase in the chemical activity (metabolism) of the egg: after fertilization it consumes oxygen and gives off carbon dioxide perhaps ten times as fast as before. Warburg has shown that the slow rate of oxygen consumption of the unfertilized egg is not much affected if the structure of the cell is destroyed by mechanical means (see page 87), but that the increased rate of oxygen consumption of the fertilized egg is completely annulled by destruction of the architecture of the cell; this seems to suggest that the increased chemical activity is not merely due to the increased permeability of the surfaces permitting oxygen and carbon dioxide to pass through faster than before. All these changes,

membrane formation, increased permeability, and increased metabolism, are part of the response of the ovum to the stimulation of the sperm's entry, and can be evoked by other stimuli of a quite different nature.

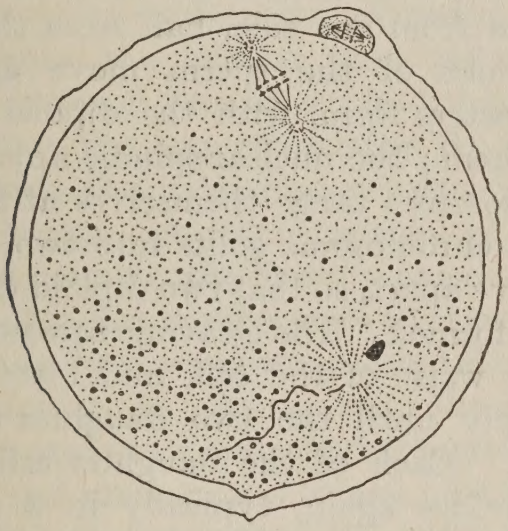


FIG. 5. Fertilization of an animal ovum, magnified 250 times.

The ovum has formed an irregular fertilization membrane; within the yolk-laden cytoplasm of the vegetative pole can be seen the sperm—the long flagellum, the dense nucleus, and the centriole about which an aster has formed. At the other pole the first polar body has been extruded, and is dividing; the nucleus of the ovum is also dividing to form the second polar body.

Once inside the cytoplasm of the ovum, the sperm begins to break up into parts. The long tail, often nipped off at the periphery of the egg cell, seems simply to degenerate and disappear. The centrioles in the middle-piece exert their curious influence on the cytoplasm of the ovum,

and build up an "aster." The nucleus swells a little by imbibing fluid from the cytoplasm, and moves over to join the nucleus of the ovum as soon as the maturation divisions are completed. The two haploid nuclei join together to form a diploid nucleus, in which half the chromosomes are derived from one side, half from the other. The centrioles of the sperm move apart, so that two asters form, with the diploid nucleus between them; the nuclear spindle appears, and the chromosomes range themselves at its equator; each chromosome splits into two, and the halves move apart, so that two diploid daughter-nuclei are formed. The cleavage furrow appears round the waist of the cell, and bites in ever more deeply until the two daughter-cells are separated. Each of the daughter-cells immediately divides again, typically in a plane at right angles to that of the first division; the four-cell stage is attained; and so the process of division goes on and on, till at last through many stages the whole highly-differentiated body is built up.

*The divisions of the egg-cell often take place in a very uniform way, determined by the structure of the unfertilized ovum.*

Although the ovum is usually spherical in shape, it is not perfectly radial in its structure—that is to say, the various visible components

of the cell are distributed not evenly or at random, but in a more or less regular arrangement. Typically, the nucleus lies rather towards one end of the cell, the *animal pole*, while the cytoplasm is most heavily charged with yolk at the other, the *vegetative* pole. It is usual to find that the polar bodies are extruded from the oöcyte near the animal pole, as we should expect, while on the other hand the spermatozoön enters the egg at or near the vegetative pole. From this point it makes its way upwards to the equator, where the nucleus of the ovum comes to meet it; the first " plane of cleavage " or plane of separation between the two daughter-cells is usually a meridian, cutting through both poles, and so is the second cleavage-plane. Different groups of animals differ greatly in the amount and arrangement of the yolk within the ovum, and the course of the subsequent cell-divisions is modified accordingly.

These relations have been most carefully worked out in the studies of E. B. Wilson and of Conklin on " cell-lineage "—studies designed to show what parts of the embryo and eventually of the adult arise from the first-formed daughter and granddaughter cells, the " blastomeres," as they are conveniently termed. Most of this work relates to certain invertebrate animals, but in a modified form the results have been shown to have a very general application, and conse-

quently we may study these best-known cases with some certainty that the principles revealed, at least, are very far-reaching. It turns out that in normal development the cleavage-planes fall so regularly into given positions, defined relatively to the two poles of the egg, that it is possible to point to a definite part of the cytoplasm of the unfertilized or newly-fertilized egg-cell and say: this part is going to produce the ectoderm, or outer layer and nervous system of the adult body; this part is going to produce the endoderm of the internal organs and alimentary canal. But of course this is a very long way from the standpoint of those earliest embryologists who thought that the egg-cell contained a miniature replica of the adult body, which had but to unfold and expand and grow. It is even too much to say, with embryologists of a somewhat later day, that the egg-cell contains definite "organ-forming substances." It is only possible to conclude that the divisions of the egg-cell normally take place on so regular a plan, that one region of the cytoplasm is found nine times out of ten to enter into the formation of one definite part of the body. Too much stress should not be laid on this early differentiation within the egg; it is sometimes possible to disarrange the visible orderliness of the ovum very considerably, and yet obtain a normal embryo; in other cases a disarrangement of the

egg cytoplasm may lead to the production of the most curious monsters, inevitably very short-lived, with their ectoderm inside and their endoderm outside! It is sometimes possible to get a complete embryo to develop from one of the first two or four blastomeres, separating it from its neighbours—at later stages differentiation has gone too far, and one cell will not reproduce the whole body, except in the very simplest animals. Let us here recall the startling experiments in which a simple animal, such as a Sponge, is rubbed through a sieve into fragments of only a few cells each; yet these fragments may join together and by a series of transformations mould themselves into a replica of the original structure of the body.

In some cases, especially in certain insects, the egg-cell may be slightly irregular in shape, so that it is possible to recognize a "front" and a "back," an "upper" and a "lower" surface, in fact an orientation in space corresponding to that of the adult. Typically, however, it is only possible to distinguish the two poles, animal and vegetative, in the ovum (it may be worth remarking that the two ends of a bird's egg do not correspond to the poles of the ovum). This polarity is frequently manifest in the orderly arrangement of the visible granules and other inclusions in the cytoplasm; but this visible orderliness is only the result of a subtler, invisible

polarity, which may persist after the granules have been disturbed and disarranged. It has sometimes been possible to show that the polarity depends on the position of the oöcyte in the ovary ; the vegetative pole is the part of the cell which is in closest relation to the maternal tissues. In connection with this problem of polarity a suggestive line of thought has been developed by Child, who points out that the animal pole is the region of greatest chemical activity, with accompanying greatest permeability and susceptibility to external changes, and is probably electrically negative to the rest of the protoplasm ; there is, as he says, a " physiological gradient " within the cell, one end being more active than the other. Nor is the appearance of such a gradient confined to the ovum, nor characteristic only of single cells : most living organisms show an " axial " gradient, the anterior or head end being the region of greatest physiological activity.

It may be desirable to insert here a brief parenthetical survey of the question of chemical correlation between cells, as distinct from the electrical correlation discussed in the last chapter. It is now a familiar fact that there are certain organs, the " endocrine glands " in the body of the higher animals, whose function it is to elaborate specific chemical substances which, carried by the blood, regulate the activity

of other tissues in various parts of the body. Of these "hormones" some are released in quantity in response to various stimuli, and their action is rapid, transient, and often striking —causing certain muscle cells to contract, certain gland cells to secrete, and so forth: while others, less sharply defined, appear to regulate growth, differentiation, or activity throughout the body during the whole or a great part of its life. The chemical nature of some of these substances is reasonably clear, and they do not show the complexity and specificity of proteins, the instability of enzymes or the elusiveness of vitamins, but are relatively simple and stable, and apparently related in structure to molecules (amino-acids, notably) already known to be present in the body. More recently it has been found that "hormones" are of great importance in the life of plants, since the differential regulation of growth by which the plant adjusts itself to the stimuli of light, gravity, or injury is brought about by the diffusion of specific substances. The beautiful work of Spemann and others on the development of Amphibia shows clearly that at a certain stage the differentiation and destiny of the cells of the young embryo are determined by chemical substances spreading from a definite region, well called the "organizer." Further evidence of the importance of chemical correlations in development and differ-

entiation in animals is given by experiments on "tissue culture": it is found, for instance, that if cells from the kidney are removed and grown by themselves in a suitable medium, their descendants tend to *dedifferentiate*, to lose their special characteristics and become generalized; but this retrograde trend may be reversed if connective-tissue cells are added to the culture. In all these cases, unfortunately, the chemistry of the substances involved is still completely obscure.

It has already been mentioned that there are few exceptions to the rule that the plane of the first cleavage of the fertilized egg-cell passes through the two poles, animal and vegetative; and its actual position seems to be further fixed by the point of entry or the subsequent path of the sperm nucleus. Quite often the two first blastomeres correspond to the right and left sides of the future body; but there are many exceptions to this, even within one species. To follow the course of subsequent cleavages in any general way is impossible; let us rather restrict ourselves to the examination of one particular type, one more or less applicable, indeed, to a large number of invertebrate animals.

It may be convenient to liken the cell to the earth, the animal pole being in the northern hemisphere; it is here that the polar bodies are extruded. The sperm enters in the Antarc-

tic, and makes its way up towards the Equator, where it meets the female nucleus near the centre. The nuclei unite, and the product divides; one daughter-nucleus moves towards the surface near Panama, the other towards Ceylon; the furrow which divides the cell into two runs along the meridian of Greenwich and down the Pacific on the other side; the first two blastomeres are the Old World and the New. It is clear that though the cells are separated by complete plasma membranes, yet there is some controlling intercourse between them: for if they are shaken slightly apart, a double monster of the "Siamese twin" type may be formed, and complete separation may lead to the production of two complete individuals. The next cleavage is also a meridian of longitude; it divides the New World down its length in the longitude of the Mississippi, and the Old World in the longitude of Calcutta: this brings us to the four-cell stage. Sometimes the two first blastomeres do not divide exactly alike, so that the second cleavage is not exactly a plane through the sphere, but it approximates to that condition.

The next cleavage is on quite a different plan; the four cells divide to form eight, but the eight blastomeres are of very unequal size. The cleavage furrow runs latitudinally round the globe, corresponding to the Arctic Circle: the

four cells towards the animal pole ("North") are small, those towards the yolk-laden vegetative ("South") pole are large: the upper four cells form the "first quartet." Next comes another latitude-cleavage, dividing the four large cells at the vegetative end; it corre-

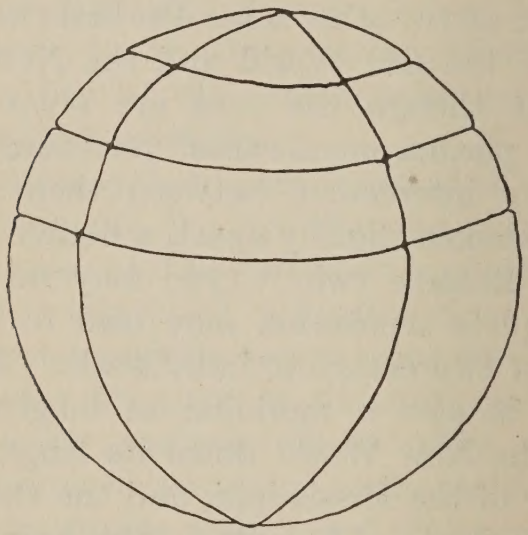


FIG. 6. The sixteen-cell stage in the development of a typical invertebrate. Nine of the twelve quartet cells can be seen at the top.

sponds to the Tropic of Cancer, and separates another group of four small cells (the second quartet) from four cells, still large, towards the south. These southern, vegetative cells divide yet again in the same direction, round the Equator, separating the four cells of the third quartet from the four cells, even now larger than any of

the others, in the vegetative hemisphere; this is the sixteen-cell stage. By this time the prospective destiny of these groups of cells is determined; the twelve upper cells of the three quartets go to form the ectoderm of the embryo, eventually of the adult; one of the four large lower cells, that which includes South America, so to speak, is particularly big; from it is derived the endoderm and also part of the mesoderm, which lies between the two other layers, and to which "quartet" cells may also contribute. There is no object in pursuing the examination further; the ball of cells continues to divide, and forms at length a hollow sphere of cells, the *blastula*; all this time, be it noted, there has been division and differentiation, but no growth in size—the weight of the blastula is a little less than that of the egg-cell, for it has used up some stores of food without replacing them. The lower, endodermal layer of cells of the blastula gets pushed in to form a cup with a double wall, as we may make a cup by pushing in one side of a punctured rubber ball; the endoderm is now inside the ectoderm, and the opening of the cup becomes small as growth continues. At this stage the developing embryo is called a *gastrula*, and here we may leave it, for specific differences begin to make it impossible to draw general inferences; so far this description, detailed though it is, is of very wide application.

One interesting point still deserves mention. Boveri, one of the most illuminating of embryologists, discovered in studying the cleavages of the egg-cell of the horse thread-worm, *Ascaris* (remarkable in having only two chromosomes), that in the nuclear divisions one of the daughter-cells regularly lost a certain amount of chromatin; it escaped into the cytoplasm and disappeared. The other cell, on the other hand, retained all the chromatin distributed to it in the usual way. This went on up to the sixteen-cell, where there was one cell left with its full complement of chromatin; the others had all lost some by "diminution." The cell which had all its chromatin then sank from the surface, and was traced onwards through many divisions in which no further diminution occurred, till it had created a whole family of cells each of which had a full chromatin complement; this family of cells formed the genital gland, and from them were descended subsequently the oöcytes and spermatocytes; so that the gametes got the full amount of chromatin that was their share, while the cells of the body had all lost part of their inheritance. This discovery would be of great importance if it had been found to be of general application. It does seem to indicate that the germ-cells have some use for chromatin which the "somatic" or body-cells have not.

*An unfertilized ovum may divide and develop, sometimes naturally, sometimes in response to an artificial stiumlus.*

At the end of last century Loeb discovered chemical means of stimulating the unfertilized ovum to its characteristic response, the formation of a membrane, the increased chemical activity, and finally division and differentiation to produce a healthy, normal embryo. Since that time the number of ways of obtaining this result has been greatly multiplied; though ova of different species vary in their willingness to start this process of *parthenogenesis* ("fatherless development"). A few examples of the means employed may be given: treatment for a short time with solutions of different concentration to sea-water, or of a different mixture of ions, or containing suitable acids—followed by a return to the normal medium; electric shock; or puncture of the surface with a needle, followed by a somewhat mysterious treatment (washing with blood) designed to repair the injury: all these lead to parthenogenesis. The reader will at once observe that every one of these methods is a *stimulus* in the general sense discussed in the last chapter, calculated to produce a temporary increase in permeability. For this reason it may be said that the ovum is an irritable cell, whose response to stimulation of a

suitable kind is to form a fertilization membrane and divide and differentiate on a pre-ordained plan. The sperm, penetrating the surface, is the natural stimulus; the other function of the sperm, which is to bring in the nuclear material from the paternal side and restore the diploid number of chromosomes, is not indispensable.

It would naturally be expected that the individuals formed by artificial parthenogenesis would be haploid in all their cells, but it is rather uncertain whether this is so. It certainly is in some cases in which parthenogenesis takes place naturally. The best instance of this is the honey-bee; fertilized eggs develop into workers (female) or under suitable conditions into queens: they have the diploid number of chromosomes. Unfertilized eggs, such as are sometimes laid by worker-bees, develop into drones (male), which are permanently haploid; it is found, naturally, that while the oöcytes of the diploid females undergo reduction-division in the usual way to form the haploid ovum, there is no reduction in the spermatocyte-divisions, since the cells are haploid already.

On the other hand, there are a great many cases in which parthenogenesis naturally, indeed normally occurs, but in which the development begins before maturation of the ovum is really over, so that the embryo is diploid from the start. There is no reduction at all: and such

oöcytes are never fertilized, perhaps cannot be fertilized: it is only by analogy that we can regard these cells as egg-cells at all. They give rise to females, which parthenogenetically give rise to other females, and so on: in a few of these species males are never found at all. But it more usually happens that at a change of season or change of diet there comes a change of habit; some of the parthenogenetic eggs develop into males, others into females which produce true eggs; fertilization takes place in the usual way, and is followed by development. There may be a number of "sexual" generations, then parthenogenesis sets in again. An example is to be found in the green-fly of rose-bushes, in which there are successive parthenogenetic generations of females all summer, but when the colder weather sets in sexual forms appear.

Nothing is known of the reason why these eggs should be independent of the stimulus of the entry of the sperm; but a curious connecting link between parthenogenesis and normal sexual reproduction with fertilization is furnished by one of the thread-worms, *Rhabdites*; here, as Hertwig showed, the egg does not develop unless the sperm enters the cytoplasm; but there is no union of the nuclei—the sperm nucleus simply breaks up; the nucleus of the ovum retains the diploid number of chromosomes and divides by itself. The function of the sperm is a

single one : to supply a stimulus causing the egg to divide ; the other function of the sperm is lost. The corollary to these cases of parthenogenesis is supplied by the experiments of Delage on " merogony " : he cut up unfertilized egg-cells, and obtained pieces containing no nucleus ; these pieces could be fertilized by the sperm and behaved like typical egg-cells except that the only nucleus within them was that of the sperm ; they formed a membrane, divided, and developed in a normal way to form rather dwarfed individuals.

*In plants there is a characteristic alternation of haploid and diploid generations, respectively sexual and asexual.*

From the animal kingdom we turn to the plants, to find there a very different order of things ; the life-history is more complicated. It is perhaps easiest to begin by considering one of the more typical ferns. The cells of the ordinary green fern plant are found to be diploid ; but on the backs of some of the leaves may be found little brown lumps, which are cases filled with *spores*. The spores are single cells, and they are formed by a reduction division, so that they are haploid ; when they ripen, they are scattered far and wide. Each spore gives rise, not to a typical fern plant, but to a very small green leaf-like growth called the " pro-

thallus." All the cells of the prothallus are haploid; from it there arise the gametes (it is therefore called the *gametophyte*, in contrast to the more familiar *sporophyte*). Small organs appear on the prothallus, the "antheridia" which produce the spermatozoa, not so very much unlike those of animals, and the "archegonia," each of which contains a single egg-cell. The spermatozoa swim (for they can only move in water) to the archegonia; there is clear evidence that they are attracted to the right direction by a secretion of a specific chemical substance. Within the archegonia fertilization takes place, in the usual way, and the egg-cell divides and differentiates to produce at length the large familiar sporophyte, while the prothallus or gametophyte dies away. By means of fertilization the diploid number has been restored.

In the flowering plants the gametophyte generation has almost disappeared; so much so, that to any but the most careful observation there appears to be no alternation of generations at all, and the temptation to liken the pollen-grains to spermatozoa is strong. But the actual course of events is rather more complicated than this. Each pollen-grain consists of two cells, closely apposed; they are formed by a reduction-division, so that they are haploid, whereas the sporophyte part of the plant, the part which

every one knows, is diploid. It still looks as though the pollen-grain was a haploid gamete, such as those met with throughout the animal kingdom ; this is almost the case, but not quite : a vestige of the gametophyte generation still survives. The pollen-grain is blown by the wind or carried by an insect, or in some way or other comes to the female part of the flower, to rest on the " stigma," the top of the pistil. Of its two cells, the larger (called the vegetative) then undergoes a peculiar change : it starts to form a long, slender tube, which penetrates into the substance of the pistil, down and down into the ovary beneath. Down this tube, as it forms, travels the other nucleus of the pollen-grain, called the generative nucleus ; and as it goes, it divides into two. These three cells, two of them hardly more than bare nuclei, are all that is left of the male gametophyte.

The female gametophytes, which are at the foot of the pistil, are rather more complex. They begin with a group of four nuclei, which are formed by a reduction-division and are consequently haploid. Of these four three disappear and one remains—the " primary nucleus of the embryo-sac." This divides and subdivides to form eight haploid cells, which constitute the female gametophyte of the higher flowering plants. These eight cells lie in the " embryo-sac," a cavity within the ovary of the flower. Three of

the eight lie at the foot and are called "antipodal cells"; two others occupy a central position, and are called "polar nuclei"; either before or after fertilization they fuse together to form a diploid nucleus. The other three cells of the eight are at the top of the embryo-sac, where there is a little canal leading in, with which the pollen-tube connects; one of these three is the egg-cell itself, the other two ("synergidæ") have some rather mysterious part to play in helping fertilization.

When the pollen-tube reaches the mouth of the embryo-sac, the two generative nuclei pass down and in, through one of the synergidæ. One of the male nuclei unites with the nucleus of the egg-cell to form a diploid nucleus, from whose divisions and development the large sporophyte will subsequently be formed. The other male nucleus goes on to meet the diploid nucleus formed by the fusion of the two polar nuclei, and unites with this to form a nucleus having *three* times the haploid number of chromosomes; this is the "endosperm nucleus." The division of this nucleus forms the endosperm, a nutritive tissue within the seed, from which the embryo itself draws nourishment. The seed, then, contains the embryo, formed from the union in fertilization of one of the male generative nuclei with the egg-cell, and consequently *diploid*; the nutritive endosperm, derived from the union of

the second male generative nucleus with the two female polar nuclei, and consequently *triploid* throughout; and the seed-coat, which is formed by the cells of the *sporophyte* which surrounded the embryo-sac. It is characteristic of seeds that after a certain number of cell-divisions, both in the embryo and in the endosperm, there comes a period of quiescence; the seed is now set free, and if it comes to a suitable place it "germinates," sooner or later; that is, it recommences the chain of divisions; the endosperm and the seed-coat disappear as they are used up, and the whole of the structure of the flowering-plant as we know it is derived from the single diploid cell formed by the union of the first generative nucleus with the egg-cell.

In the flowering plants, then, the haploid gametophyte generation has become a mere vestige; in the male form there are but three nuclei all told, in the female form eleven; and two of the nuclei in the first case, three in the second, are destined to fertilization. The gametophyte of the higher plants is almost comparable to a parasite on the sporophyte, it is so small, so dependent on its host, so degenerate. It is clear that we approach here the condition typical of animals, in which the number of haploid cells is very small, and all of them except the three polar bodies are intended to take part in fertilization. Strasburger's suggestion that there

is an alternation of generations in all animals, in which the haploid generation is represented

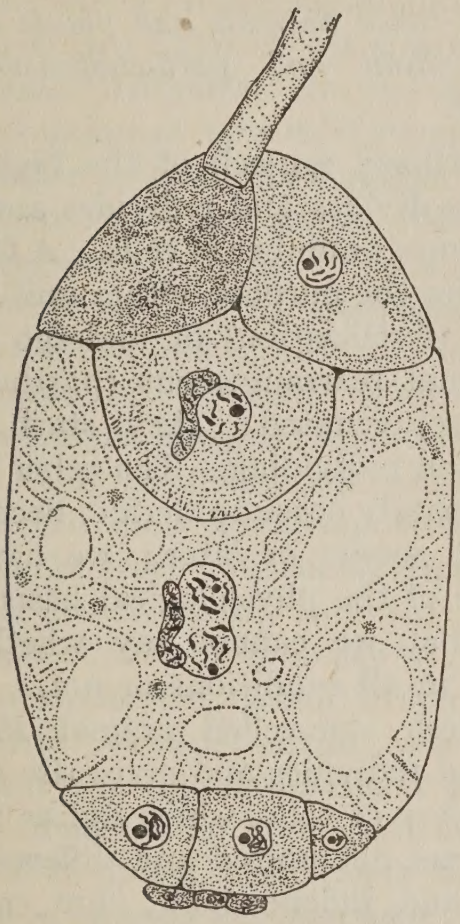


FIG. 7. Fertilization in a flowering plant, 300 times magnified.

At the top the pollen-tube is seen entering one of the two synergidæ, which has disintegrated. Below this is the egg-cell, and one of the twisted generative nuclei of the pollen-grain is applied to the ovum-nucleus. Around and below this is a large cell, from which the endosperm will arise; its cytoplasm is filled with inclusions of all kinds, and at the centre the two polar nuclei are seen partially fused, with the second male generative nucleus beside them. At the foot of the embryo-sac are the three antipodal cells, and below them the three degenerate members of the original group of four haploid cells.

only by the gametes and polar bodies, is perfectly logical, but not particularly illuminating.

*In the one-celled animals and plants analogies to fertilization and parthenogenesis may be traced.*

The fertilized egg-cell of the higher animals and plants divides and redivides and gives rise to an enormous number of cells. A few of these, set apart for the purpose, either unite with some other cell (fertilization) or undergo some complex internal rearrangement (parthenogenesis), and are rejuvenated; they start life anew, dividing and redividing. The other cells of the body ultimately cease to divide, become "old," and perish sooner or later; we have so little knowledge of the physiological basis of old age in cells that we cannot say whether this is inevitable in all conditions or not.

The simple one-celled animal *Paramœcium* divides and redivides and gives rise to an enormous number of cells, which unlike the cells of the body are not united and differentiated but all alike and independent; but, as Bütschli pointed out fifty years ago (1876), the division of the fertilized egg-cell to form the body, and the division of the Protozoön or one-celled animal to form myriads of individuals, are perfectly comparable processes. The divisions of the Protozoa are at last interrupted; there

comes a stage at which either union with another cell (conjugation) takes place, or there is a complex internal rearrangement (endomixis); these processes are followed by a new cycle of straightforward divisions. It is natural to compare the union of two cells in conjugation with the similar union in fertilization, the internal rearrangement in endomixis with that of parthenogenesis; yet it should be noticed that while only a few of the cells descended from the ovum are capable of either fertilization or parthenogenesis, *all* the cells descended from one Protozoön are capable of conjugation or endomixis. The ultimate loss of the great majority of the cells descended from the fertilized egg is the price paid for differentiation in the building of the body.

The process of conjugation between individual one-celled animals and plants is complex, and extraordinarily variable from one type to another; only a few examples can be considered. Belar has studied the conjugation in the sun-like Protozoön *Actinophrys*: in the ordinary way it multiplies by orderly division into two similar cells, which become independent individuals in the usual way; at times, however, this process of multiplication by division is interrupted. Two daughter-cells, formed by a typical division, do not separate but remain closely apposed. In each one the nucleus divides twice, and the

first of these divisions involves a reduction of the chromosomes from diploid to haploid. Three of the four haploid nuclei in each cell are cast out and perish, recalling the polar bodies of the oöcyte or the three cells which are lost in the formation of the embryo-sac in the flowering plants. There remains one haploid nucleus in each cell; these unite together, the cytoplasms unite, and a new diploid individual is produced, which starts a new series of ordinary divisions. Except that the two uniting cells are sisters, this closely resembles the process of fertilization in animals; the reducing division immediately precedes the union of the cells.

The simple one-celled green plant (alga) *Spirogyra* forms long threads of cells, which remain united together end to end, but are otherwise independent and all similar; they divide and subdivide in the usual way. At times, however, conjugation takes place: two filaments come to lie side by side, and a union is established between the cells of the one and the corresponding cells of the other. From one of the filaments, which thereby becomes empty, all the nuclei and cytoplasms flow across to unite with the cells of the other filament in pairs. The number of individuals and number of nuclei is thereby halved, and each nucleus will have double the usual number of chromosomes, since no reducing division preceded the union of the nuclei.

But a reducing division immediately sets in, restoring the usual number of chromosomes; it takes place in the typical way, a double division of which the first involves reduction, followed by the degeneration and disappearance of three out of the four nuclei. It is clear that *Spirogyra* is exactly the opposite of the higher plants and animals; the usual form is haploid, the diploid nucleus only persists for a very short time after each conjugation; reduction follows union, instead of preceding it.

Conjugation in the actively-swimming slipper-shaped Protozoön *Paramœcium* is rather more difficult to follow. There are two nuclei in each cell, one large and one small, and when the cell divides into two in the ordinary way these nuclei divide separately, and half of each goes to each daughter-cell. In conjugation two individuals (*not* sister-cells nor of near kin) come together side by side, and in each one a process goes on recalling the preparations for union in *Actinophrys*. The large nucleus breaks up into fragments and disappears; the small nucleus divides twice, probably with a reduction of the number of chromosomes from diploid to haploid, and three of the four nuclei produced perish. The survivor in each cell divides again, into two slightly different parts; one is called the "male" or "migratory" nucleus, the other the "female" or "stationary" nucleus. The

migratory nucleus from each cell starts to move across into the neighbouring cell, where it unites with the stationary nucleus; there is an exchange of migratory nuclei between the two partners in conjugation. No permanent fusion of the cytoplasm takes place, and when the exchange of nuclei is completed the two mating cells separate and independently start a process of division, in which the large nuclei are regenerated and the normal state is restored. Here again we have reduction immediately preceding union of the nuclei, but we have no union of cytoplasms. It is in *Paramœcium* and its allies that endomixis has been most studied; this is a rearrangement of nuclear material within the cell, involving the temporary disappearance of the large nucleus and much activity on the part of the other, with formation of small daughter-nuclei which degenerate; but the meaning is not well understood and it is a difficult subject for chromosome study.

Finally, it should be stated that there are many forms in which conjugation takes place, not between cells of the ordinary type, but between rather unusual cells which arise from time to time; and sometimes the two partners in conjugation are not similar but unlike, so that one may be said to be male and the other female. Moreover there are forms, especially in the bacteria, in which conjugation never seems

to take place at all, and it is not known if any rhythmic process of internal re-organization takes place; some of them have no visible nucleus. There are many theories of the significance of conjugation and endomixis—so many that their discussion cannot be undertaken here. It does seem that conjugation or endomixis gives the cell a fresh start, a rejuvenation, as fertilization or parthenogenesis gives renewed youth and vigour to the ovum; but even this is debated, and the mechanism of the process is hopelessly obscure. We shall never know much about rejuvenation unless we discover the real meaning of old age from the chemical and physiological aspect.

*The independence of characters is a fundamental principle in heredity.*

In the previous discussion of the cleavage and differentiation of the fertilized egg-cell, attention was given only to the very broadest aspect of the problem why like produces like; why the sea-urchin egg develops to form ultimately another sea-urchin, and so on; and it was implied that a part at least of the answer must be searched for in the differentiated structure of the cytoplasm, or perhaps the interaction of nuclear material and different parts of the cytoplasm. We may now turn to individual differences, to variations within one species—

the finer, narrower aspect of the problem of like begetting like. The first hints of an answer to this question, still more than obscure to-day, were given by the experiments of Mendel in 1865—famous now, though unnoticed for thirty-five years.

Mendel's laws of inheritance are best studied by example. He bred together two varieties of one species, a tall pea and a dwarf pea. The first generation (called the $F_1$ generation) of the offspring of this mating were not of medium height, as might have been expected; they were all tall; there was no blending in this case, though sometimes there is. These hybrids were then bred together, and the second ($F_2$) generation was examined; again there were none of medium size; some were dwarf, but three times as many were tall. It was evident that the power to produce dwarfs had lain latent in some at least of the tall peas of the $F_1$ generation. Now if the dwarf peas of the $F_2$ generation were bred together, the next ($F_3$) generation were all dwarfs, and so on for many generations; these dwarfs had no latent power to produce tall peas; they were dwarfs through and through and as long as they were mated together could produce only dwarf peas.

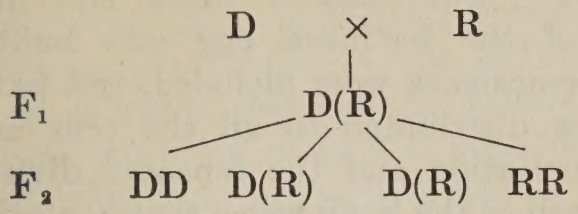
The explanation suggested by Mendel for these facts was simple and convincing. He suggested that to the hybrid the tall parent supplied an

element for tallness, the dwarf parent an element for dwarfness. In the $F_1$ generation, both these elements were present, yet all were tall (and it is of no importance in this case whether the tall parent is the male or the female), so the element for tallness is said to be "dominant," meaning that it overpowers the "recessive" element for dwarfness when both are present. Mendel went on to suggest that although the $F_1$ generation were all tall in aspect, yet the element for dwarfness was there all the time; and further, that the germ-cells of the $F_1$ generation were of two different kinds, in equal numbers: half carried the element for tallness *only*, half only the element for dwarfness. Now if these germ-cells united together in pairs (in fertilization) and at random, it is easy to see what would happen: some of the male cells that carried "tallness" only would meet with female cells also carrying "tallness" only, and the cell formed by their union and the plant that arose from it would be tall through and through and have no element of dwarfness; on the other hand, if the union of the cells took place at random, an equal number of the male cells that carried "tallness" would meet with female cells carrying "dwarfness," so that the plant which arose from this union would have both characters—the dominant tallness predominating, however, as far as outward appear-

ance went. The same kind of plant would arise from the union of a male cell carrying "dwarfness" with a female cell carrying "tallness," a plant having both characters, with the dominant tallness in its outward aspect. On the other hand the union of a male cell carrying "dwarfness" with a female cell also carrying "dwarfness" would produce a plant that was dwarf through and through, with no tallness hidden anywhere within it; it would "breed true" as a dwarf for ever; the recessive character of dwarfness had been extracted from the mixture.

Let us now count up, remembering always that half the germ-cells of each sex carry "tallness" and half "dwarfness" and that they mate at random. There will thus be four kinds of union, which may be called tall-and-tall, tall-and-dwarf, dwarf-and-tall, dwarf-and-dwarf, and there will be equal numbers of each kind. But tall-and-dwarf and dwarf-and-tall are identical, as it happens, as soon as the union of the nuclei has taken place in fertilization; and these mixed forms will all be tall in appearance, since tallness dominates over dwarfness; the latent dwarfness is only revealed, as in the mixed $F_1$ generation, by breeding these forms together. The net result is that tall and dwarf forms appear in the $F_2$ generation, and there are three times as many tall plants as there are dwarfs. The following diagram summarizes the position,

where " D " stands for the dominant character, " R " for the recessive (dwarfness), which does not show in the presence of the dominant.




Clearly the germ-cells of the " DD " group can only contain the dominant character, and will breed true if mated among themselves; the " RR " forms also breed true; the " D(R) " forms, though outwardly similar to the " DD " forms, behave if inbred like the $F_1$ generation, and yield once more three dominant forms, two of them mixed, for each recessive.

When this work of Mendel's was rediscovered in 1900, its possible connection with cytological studies was at once apparent. Boveri had already shown that in the fertilized egg-cell and in all the cells arising from it half the chromosomes were derived from one parent, half from the other. In the maturation-process, however, reducing divisions take place, and of each pair of chromosomes one member passes into each of the daughter-cells. from which the gametes themselves are derived. If then the material basis of the character, chemical or physical, whatever it might be, was contained in the

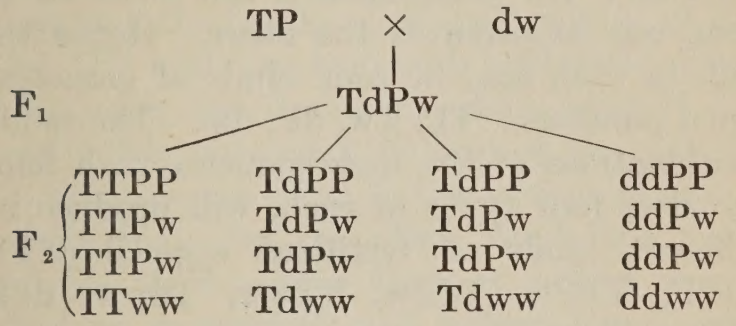
chromosomes, much was explained. One chromosome of one of the original parents carries "tallness," the corresponding chromosome of the other "dwarfness"; when the diploid nucleus of the fertilized egg was built up, both chromosomes were included, and parts of each were distributed to all the cells arising by differentiation and the repeated divisions; in every cell of the body was a representative of the "tallness" chromosome, and also a representative of the "dwarfness" chromosome from the other parent; but in some way or other the dominant character overruled the recessive, and the recessive character remained concealed. In the formation of the germ-cells, however, at the time of the reducing divisions, this pair of chromosomes is separated; the "tallness" chromosome goes to one daughter-cell, and thence to half the gametes; the "dwarfness" chromosome to the other daughter-cell and the rest of the gametes. In each haploid nucleus, such as those of the gametes, there is either the "tallness" or the "dwarfness" chromosome, but not both. This corresponds so well with the results of Mendel's experiments that the view that the chromosomes carry the Mendelian characters in some unknown material form (to which the name "gene" is applied) is now very generally accepted, and has been greatly extended in directions shortly to be described.

The meaning of the words " the chromosome carries the factor for tallness," and so on, still remains quite obscure; we cannot in the least conceive how some material substance in the chromosome so influences the growth of the plant as a whole that tallness results from its presence. In a few cases it is possible to make guesses, which may or may not be near the truth. For example we may consider the case of white " albino " rabbits, whose whiteness is shown to be a recessive to the dominant dark type. Chemically the dark pigment is known to be produced by an oxidizing enzyme *tyrosinase*, and albino rabbits are known not to have any tyrosinase. If we suppose that the chromosome of a dark rabbit contains tyrosinase, and that of a white rabbit does not; and further that the enzyme reproduces itself in some way, and cannot be formed by cells which have not received a trace of it in their chromosome complement; then plainly the albino character is recessive, for a cell which has received from *one* parent (even if not from both) the minute trace of the enzyme required will be able to manufacture the dark pigment; but these are large assumptions to make. The view taken here, that the recessive character is due to the *absence* of something from the chromosome, is called the " presence-and-absence " theory; it works well up to a point, but there are many difficulties

attached to it. Goldschmidt especially has brought forward cases in which the *quantity* of a factor inherited seems to be significant.

Mendel's second great contribution to the subject was to examine cases in which two quite different characters were involved. He had found that the character " purple flowers " was dominant to the character " white flowers." If white and purple were crossed, the $F_1$ generation was purple, the $F_2$ generation produced whites (extracted recessives) but they were outnumbered by the purples by three to one; this is just another case of what we have considered above in the characters " tall " and " dwarf." But now the question arose, what happened when " tall, purple " peas were crossed with " white, dwarf " peas. In the $F_1$ generation, as was natural, only the dominant characters appeared; all were tall and purple. In the $F_2$ generation a most bewildering mixture of forms turned up; there were many tall purple, fewer tall white, the same number of dwarf purple, very few dwarf white. What had happened was this: the two characters distributed themselves quite independently. Of every four tall ones, three were purple and one was white—the usual ratio; of every four purple plants, three were tall and one was dwarf—the usual ratio again. Out of every sixteen members of the $F_2$ generation, nine were tall and purple (both dominant

characters), three tall and white and three purple and dwarf, and one was dwarf and white (both recessive characters). This is made clearer by a diagram in which " T " is tall and " d " is dwarf, " P " is purple and " w " is white—the recessives being written in small letters.




Examination of this diagram will show that, since the recessive characters do not show if the corresponding dominant is present, there are nine tall purples (of four kinds, TTPP, TdPP, TTPw, TdPw), three tall whites (TTww and Tdww), three dwarf purple (ddPP and ddPw) and one dwarf white (ddww). Here again the chromosome theory furnishes an explanation ready-made. Let us simply assume that these different characters are on different chromosomes; then in each diploid nucleus of the $F_1$ generation, from the fertilized egg-cell onwards, there will be four important chromosomes; one carrying " tall " and its synaptic mate carrying " dwarf," one carrying " purple " and its synap-

tic mate carrying "white." Now at the reduction divisions these synaptic mates will be separated; but it does not follow that the two pairs will always separate in the same way. In one spermatocyte "tall" and "purple" may go together, in another "tall" and "white" together; the combinations are made at random, one as often as the other. Hence there will, in each sex, be four kinds of gametes in equal numbers: TP, Tw, dP, dw. The random combinations of the male gametes with female gametes, four types of each, will produce nine different kinds of fertilized egg-cell (TTPP, TdPP, TTPw, TdPw, TTww, Tdww, ddPP, ddPw, ddww), but owing to the dominance of the characters "T" and "P" only four types of plant will be recognizable in the $F_2$ generation, and they will be present in the ratio 9 : 3 : 3 : 1; anyone can work this out for himself. The point is that two different pairs of opposing characters vary independently of each other. Mendel extended this to *three* pairs of characters, but the principle is the same, and the demonstration more complex.

*It is demonstrable that sex is inherited on Mendelian principles.*

So far we have considered only two kinds of crosses for one pair of characters, the crossing of pure dominant with pure recessive, DD $\times$ rr,

and the inbreeding of the resulting $F_1$ generation, Dr × Dr. Let us now consider the so-called "back-crosses," where a mixed form is bred with a pure form of either kind, as in DD × Dr or Dr × rr. In the first case, the cross of a dominant with a mixed, the gametes will be from one parent D and D, from another D and r; it follows that the fertilized egg-cells will be of two kinds, DD and Dr, but both will yield forms having only the dominant character; if the recessive lies concealed in one of the parents only, it can never come to the surface in any of the offspring. On the other hand, the back-cross with the recessive, Dr × rr, will be accomplished by gametes D and r from one parent, r and r from the other, and the egg-cells will be of two kinds, Dr and rr. Back-crosses always give equal numbers of two kinds of offspring, one corresponding to each of the parents; but only in the back-cross with the recessive are the parents or the offspring visibly different. Now it is plain that if we regard the male as a mixed form, Dr, and the female as a recessive, rr (that is to say, consider femaleness as a pure recessive character, maleness as an admixture of this character with something else), then the offspring will be Dr, male, and rr, female, in equal numbers—which is, speaking generally, the usual state of things. It is plainly permissible to say that in any fertilization the sperms are either

D or r, the ova always r, so that Dr males and rr females are produced in equal numbers; or again it would do equally well to regard the female as the mixed Dr form and the male as the pure rr form.

This would be little better than symbol-mongering if it did not have an important bearing on the chromosome theory. It has been found in a great many animals and some plants that there are chromosomes which vary in the different sexes, and undoubtedly are concerned in the determination of the sex of the fertilized egg-cell. The first type to be considered, and the simplest, is found in the house-fly and the vinegar fly and many other animals, probably including man. In the diploid nuclei of the female there are two similar sex (or "X") chromosomes; one of these is cast out in the first polar body, being separated from its synaptic mate in the preceding reductional division. In the diploid nucleus of the male there are two corresponding chromosomes, but they are not exactly similar, one being larger ("X") than the other ("Y"); at the reductional division these synaptic mates are separated, so that the X chromosome appears in half the sperms, the Y chromosome in the other half. When one of the X sperms fertilizes an ovum, the diploid nucleus formed naturally contains two X chromosomes—one from each gamete—and the organism that results is female.

When one of the Y sperms fertilizes an ovum, the resulting diploid nucleus contains an X chromosome and a Y chromosome, and the organism that develops is a male. This shows simultaneously the importance of regarding sex as a Mendelian character, and the plausibility of regarding the chromosomes as the carriers of the " genes " or Mendelian factors.

There are other types, to be sure : sometimes the Y chromosome is absent, so that half the sperms have an X chromosome and half have nothing corresponding to it—the result is the same as before ; this occurs in the dog and the cat, for instance, and is probably a modification of the previous type. In birds and butterflies, however, it seems that it is the ova that are of two kinds, while the sperms are all alike ; the female is Dr, the male rr ; but the cytological evidence is not so clear in this case. The statement that the sex is determined by the chromosome content of the egg-cell must not be taken too rigorously ; it is better to say that there is a tendency to one sex or the other, depending on the chromosomes ; for the evidence that sex may be partly determined by variations in the environment is too good to be neglected. Probably all Mendelian characters are to be regarded as tendencies in one direction or another, rather than as immovable guides.

*There are important exceptions to the rule that characters vary independently.*

Mendel found that when he crossed plants which differed in two points, the two characters varied independently, with the result that in the $F_2$ generation all the possible combinations of the characters (four, to the outward view) occurred in a given ratio: this has been discussed sufficiently above. In 1906 Bateson and Punnett found that to this rule there were exceptions; there were characters that varied not independently but together: such characters are said to be coupled or linked. If, for instance, the character of flower-colour (white or purple) was linked with the character of height (tall or dwarf) in the pea—as we have seen it is not—then the $F_2$ generation of a cross of tall purple with dwarf white would be all either tall purple or dwarf white, three times as many as the former; tall white and dwarf purple would never occur.

Linkage of characters is best known in the vinegar fly, *Drosophila melanogaster*, where it has been brilliantly studied by T. H. Morgan and his colleagues. If, for instance, a female with yellow wings and white eyes is mated to a male of the normal wild type with grey wings and red eyes, the $F_1$ generation show both the linked characters, grey wings and red eyes, if

they are females, and yellow wings and white eyes if they are males—this need not be discussed for the moment; if now these males and females of the $F_1$ generation are bred together, in the $F_2$ generation we find either grey wings and red eyes together, or yellow wings and white eyes together; the two characters are linked. Similarly if an abnormal fly with vestigial wings and a black body is mated with a normal winged fly of the wild-type colour, the $F_1$ generation are apparently normal; but if they are back-crossed with the recessive black vestigial-wing type, they yield equal numbers of two sorts of offspring, normal and black-vestigial. Black body colour and vestigial wings, normal colour and normal wings, these are linked characters.

This is readily explained by the chromosome theory. The independent assortment of pairs of characters, such as colour and height in Mendel's peas, is possibly due to the independent assortment of the chromosomes in reducing divisions, as we have seen. But if two characters are located on the *same* chromosome, they will never be separated by the pulling apart of the synaptic mates. If in the mixed ($F_1$) form above "wing" and "wild colour" are on one chromosome, "vestigial wing" and "black colour" on the synaptic mate of that chromosome, then on reduction and subsequent division

there will be two sorts of gametes, in one the chromosome with "wing" and "wild," in the other the chromosome with "vestigial" and "black." Linkage is explained by the supposition that the genes of the two linked characters are located in the same chromosome, so that the shuffling of the chromosomes in reduction does not get the characters apart.

In *Drosophila* the study of these linkages has been pushed so far that over three hundred "mutant characters" or variations have been studied, and their linkages with one another determined. They fall into four linkage groups: that is to say, the three hundred variations fall into four groups of characters which do not distribute themselves at random, but remain united in breeding. On our theory, this should mean that these characters are located on four pairs of chromosomes. It is surely more than a coincidence that there *are* four pairs of chromosomes in *Drosophila*. It may be added that in at least one other case the number of linkage-groups of characters, as determined by breeding, corresponds to the number of chromosome pairs. It was merely a chance that Mendel did not stumble on any case of linkage, for the original discovery of Bateson and Punnett was that in sweet-peas the characters purple flowers and long pollen grains, red flowers and short pollen grains were linked together, that is, they did not

distribute themselves independently in the $F_2$ generation, but tended to stick together.

Now, accepting the chromosome theory, we may say of *Drosophila* : one chromosome carries the following hundred characters, another carries these seventy-five characters, another sixty, and here is a chromosome, surely a small one, which carries only two or three characters known to us (and in fact the chromosomes of one pair in *Drosophila* are very small). But how are we to tell which chromosome is which, even in this case where their shape is recognizable and the number small ? One important means is afforded by the fact that one, the largest, of these linkage groups is on the sex-chromosome ; the characters are sex-linked (the existence of sex-linked characters, such as hæmophilia or " easy bleeding " in man, has long been known). Thus the character for eye-colour in *Drosophila* is sex-linked ; it is situated on the X-chromosome. In the male diploid nucleus, however, there is as we have seen only one X chromosome, corresponding to which is a small Y chromosome which does not bear the character. In the female there are two X chromosomes, both of which bear the eye-colour character. Consider now, as we have done already, the mating of a female with the recessive character " white eye " with a male having the dominant character " red eye." Those egg-cells of the $F_1$ generation

which yield females will have from the female side an X - chromosome - with - white - eye - gene, from the male side an X-chromosome-with-red-eye-gene, and as the red colour is dominant the $F_1$ females will have red eyes. But the $F_1$ males got from the male side only the small Y chromosome, which does not affect eye-colour, and from the female side one X-chromosome-with-white-eye-gene, and consequently, in the absence of the dominant gene, the $F_1$ males have white eyes. When these are crossed together, the $F_2$ generation have, in equal numbers, red and white eyes without distinction of sex. The whole situation is completely changed if the female original parent has red eyes, the male white eyes; then, since the dominant red-eye gene is on the X-chromosome from the female side, which is present in *all* of the $F_1$ generation whatever their sex, then all the $F_1$ have red eyes; for although one of the two X chromosomes in the $F_1$ females carries the white-eye-gene (the X chromosome which came from the male parent, and is not present in the male offspring), this is recessive and does not show except on further breeding. Here, in the $F_2$ generation, it *does* show: for half the ova have an X-chromosome-with-white-eye-gene, and if one of these meets a sperm with no X chromosome but only a Y, then the result will be a *male* with the recessive white eye colour un-

corrected; the rest of the ova and the rest of the sperms carrying a red-eye-gene, all the females and half the males of the $F_2$ generation have red eyes. This is a complicated affair, certainly, and may perhaps be made easier by a diagram.

I

| | | |
|---|---|---|
| | White-eyed female; | × Red-eyed male; |
| | One kind of ova, X-white | Two kinds of sperm, X-red Y |
| $F_1$ | X-red plus X-white gives | Y plus X-white gives |
| | Red-eyed female; | × White-eyed male; |
| | Two kinds of ova, X-white X-red | Two kinds of sperm, X-white Y |
| $F_2$ | X-white plus X-white: White-eyed female. | Y plus X-white: White-eyed male. |
| | X-red plus X-white: Red-eyed female. | Y plus X-red: Red-eyed male. |

II

| | | |
|---|---|---|
| | Red-eyed female; | × White-eyed male; |
| | One kind of ova, X-red | Two kinds of sperm, X-white Y |
| $F_1$ | X-red plus X-white: | X-red plus Y: |
| | Red-eyed female; | × Red-eyed male; |
| | Two kinds of ova, X-red X-white | Two kinds of sperm, X-red Y |

| | | |
|---|---|---|
| $F_2$ | X-red plus X-red : Red-eyed female. | X-red plus Y : Red-eyed male. |
| | X-red plus X-white : Red-eyed female. | X-white plus Y : White-eyed male. |

The inheritance of hæmophilia in man is of this second type; the recessive character appears only in the males but is inherited only through the females; careful inspection will show that this is true of the recessive white-eye character brought in by the male in the second part of the diagram, supposing the stock to be bred with a normal one.

*Characters linked together are sometimes separated.*

The phenomenon of linkage is an apparent exception, easily explained by the chromosome theory, of the second law of Mendel, that of the independent assortment of the genes—the law that characters vary independently. It remains to explain that there are exceptions to the law of linkage; characters linked together are not indissolubly united. We have seen that in *Drosophila* grey-wing and red-eye are linked characters (linked also with sex), and in mating with a different type, yellow-wing and white-eye, these linked characters stick together, so that we do not get yellow-wing and red-eye, or grey-wing and white-eye, in the $F_2$ generation. But as a matter of fact we *do* get some of these

mixtures, though very few (1½ per cent.); the linkage has, in a few cases, been broken. Such types, showing a broken linkage, are called "cross-overs;" and different linkages vary in the ease with which they are broken—that is, in the number of cross-over forms that occur. Morgan has also shown that whole blocks of characters or genes go over together.

This is most easily made comprehensible by a little anticipatory theory-building. There are many genes on one chromosome, we know; and the chromosome that is the synaptic mate of this has all the corresponding genes, whether the same identically (in pure forms) or an opposing "allelomorph" (in mixed forms), dominant or recessive as the case may be. Let us now suppose that the genes are arranged along the chromosome in linear order—the chromosomes being at one stage very long and thin, this is reasonable—then we may represent a pair of chromosomes in a mixed form in the following way:

A B C D E F G H I J K L M N
a b c d e f g h i j k l m n

Thus we may imagine that "F" is red-eye and "f" white eye, "G" grey-wing and "g" yellow-wing; the capital letters are all linked together, so are all the small letters. Now in "cross-over" cases the linkage is broken, and

when red-eye gets separated from grey-wing the position may be represented thus:

A B C D E F g h i j k l m n
X
a b c d e f G H I J K L M N

The two linkage groups have crossed over, and a whole block of genes has passed from one to the other. It is found that genes never go over singly, so that we do not get

A B C D E F g H I J K L M N
XX
a b c d e f G h i j k l m n

though we may get a fairly small block of genes crossing over

A B C D E F g h i j k L M N
X X
a b c d e f G H I J K l m n

But how in the world is all this known? The answer is quite simple: it is possible to estimate the distance between two genes (relatively, not in absolute measurement) by the amount of crossing over that takes place, as shown by breeding experiments. Red-eye and grey-wing are very seldom separated, only three times in two hundred; therefore they are close together

(F and G in our diagrams); other characters separate much more often, so often that the linkage may escape notice; they are far apart in the chain, so that when a cross-over occurs anywhere, it separates them; whereas only a cross-over at the right place can separate F from G. In this way Morgan and his collaborators have been able to draw up diagrams of the order of the genes, naming each one accurately, along the linkage groups. It is interesting that the "chromosome map" of one species may be recognizably like that of a closely related species.

It is not integral to this part of the work that the chromosomes should be the carriers of the genes, but we have already found good reason to believe that they are. There is also evidence that the crossing-over is an actual twisting of the chromosomes over each other, when they are closely apposed in synapsis before reduction takes place. The two synaptic mates, instead of lying side by side, twist over each other; when the separation comes, pulling towards the two daughter-nuclei, the chromosomes do not disentangle themselves, but *break*, so that a part of each goes to each daughter-nucleus; one end of one and the other end of the other to each nucleus. In fact, they separate just as we might separate the upper from the lower row of letters in one of the crossing-over diagrams, though in

each half we get some small and some capital letters. It must be admitted that the evidence that the process in reality accords with this theory is somewhat indirect.

We find, then, that as the theory that linked genes were on the same chromosome explained the phenomenon of linkage, so the theory that the genes are in linear order on the chromosomes and that the chromosomes twist over one another and break in synapsis explains the fact of crossing-over of linked genes. It is thus that Morgan has been able to prepare " chromosome maps " of *Drosophila*, showing the exact position of all the known genes on the chromosomes. How incredible this would have seemed twenty years ago ! The fact that the chromosomes are not very flexible and do not twist over each other readily may explain why genes go over in blocks, and why one cross-over is never very near another, so that the blocks are never less than a certain size.

One or two scattered points deserve attention. It is possible to increase the amount of crossing-over experimentally, by raising the temperature at which the flies are kept. It is found, curiously enough, that crossing-over does not occur in both the reduction divisions, but in *Drosophila* only in the female ; in general only in the sex that has the two X chromosomes, that is the sex that is the " rr " form (the male in the case

of butterflies, the female in most other cases). This does not apply only to crossing-over in the X-chromosome, which would be easily explainable, but to all the other chromosomes as well. Then again, Morgan has found cases in *Drosophila* where instead of the usual pair of very small chromosomes, there is only one, or there are three: with these changes there go slight changes in outward form (a support for the chromosome theory), but apparently this chromosome is so small that these variations are not of much significance.

There are still some who believe that, although the known behaviour of the chromosomes fits in so well with the theory that they are the carriers of the heredity, the almost unknown behaviour of the cytoplasm and its constituents may fit in even better. But there is, on the whole, general agreement as to the main principle (if not on such advanced points as the crossing-over of the chromosomes in synapsis), that the chromosomes carry the hereditary characters. We are still quite in the dark, however, on three main points: first, the nature of a gene; second, the manner in which the gene influences development; third, the way in which the variant forms or mutants of genes arise, for though crossing-over explains new combinations of characters, it does not tell us how new characters appear. It is possible to increase the number of mutants

experimentally, but not to foretell their nature. But the research of the recent past has been so brilliant, that it casts a bright effulgence on the future.

# BIBLIOGRAPHICAL NOTE

*General Cytology*, edited by E. V. Cowdry (Chicago, 1924), is the only recent work which covers the wide field of the present little book ; it is a large volume, written in symposial form by thirteen distinguished American scientists ; it contains, however, little that corresponds to the discussion of the chemistry of the cell given here in Chapters II and III. Chapters I and V of this volume deal with " pure " cytology, and in this field there are many books, the best perhaps E. B. Wilson's *The Cell in Development and Heredity* (New York, 3rd ed., 1925) ; good, also, are the books by Agar, Doncaster, and Sharp—the last-named distinguished by its emphasis on the plant kingdom. The discussion of surfaces and colloids in Chapter II is taken largely from Freundlich's monumental *Colloid and Capillary Chemistry* (London, 1926), and of many smaller works on the subject the same author's *Elements of Colloidal Chemistry* (London, 1925) is one of the best ; but as a whole Chapters II and IV are modelled on a splendid book by Höber, *Physikalische Chemie der Zelle und der Gewebe* (Leipzig, 6th ed., 1926), unfortunately not translated into English. Chapter III is pure biochemistry, and reference should be made to the text-books of that subject ; Robertson's *Principles of Biochemistry* is planned with special reference to the cell. Bayliss's *Principles of General Physiology* (London, 4th ed., 1924), a book of very wide scope, may be turned to for an expansion of the three centre chapters of this brief survey of the field.

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